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Studies of the pH of normal resting saliva. II. Diurnal variation. *Robert E. Brawley*, B.S., M.S., D.D.S. (University of Rochester).. 79

Studies of the pH of normal resting saliva. III. Effects of vitamins A and D in school children. *Robert E. Brawley*, B.S., M.S., D.D.S. (University of Rochester)..... 87

The iron content of teeth of normal and anemic rats. *Sarah Ratner*, B.A., M.A. (Columbia University)..... 89

Medico-dental case records: XII. Fusospirochetal angina. A clinico-pathological study. *Bissell B. Palmer*, D.D.S., F.A.C.D., and *Malcolm W. Carr*, D.D.S., F.A.C.D. (Fifth Avenue Hospital, New York City)..... 93

A critique of Bolk's "problems of human dentition." *M. Russell Stein*, D.D.S. (Columbia University)..... 101

Dens in dente. *Paul C. Kitchin*, M.S., D.D.S. (Ohio State University)..... 117

Cataphoretic velocity and localization of streptococci isolated from infected teeth of persons having systemic disease. *Edward C. Rosenow*, M.D. (Mayo Foundation)..... 123

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STUDIES OF THE pH OF NORMAL RESTING SALIVA. II. DIURNAL VARIATION[1]

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In a previous paper (2), the author reported data concerning the relationship of age and sex to the pH of normal resting saliva, and pointed out that in order to establish, for such saliva, an average that might be used as a standard in future investigations, it would be desirable to study further another factor: diurnal variation. Foa (5), using an electrometric method, reported the pH of normal saliva to be 8.2 before meals, 8.3 after meals. Bloomfield and Huck (1) found the diurnal variation the same as the normal variation. La Farga (7) reported saliva to be very acid in the morning, alkaline in the afternoon. Starr (11), studying the relationship of alveolar CO_2 and normal resting saliva, indicated that after the noon meal both alveolar CO_2 and salivary acidity increase. McClelland (8) noted that the pH of saliva varied in the same and different individuals throughout the day. Popper and Hoffner (9), using an electrometric method, found the pH of saliva fairly constant in each individual. Henderson and Millet (6) reported that the pH of saliva drops after meals to a point slightly below that previous to the meal, and then rises gradually between meals.

Procedure. The method (colorimetric) was outlined in detail in the previous paper (2). Determinations were made at various times from 7 A.M. to 5 P.M. Specimens were collected at least 30 minutes, in most instances an hour or more, after meals. For all subjects excepting a few infants, and adults of extreme age, the volume of the samples was 2 cc. Determinations were made on 3405 apparently normal individuals, ranging in age from 3 weeks to 101 years.

[1] Presented at the thirteenth general meeting of the International Association for Dental Research, Chicago, March 16 and 17, 1935.

TABLE 1

Average pH of normal resting saliva of males and females at various hours during morning and afternoon, arranged in age groups

AGE-GROUP	HOUR	MALE		FEMALE		TOTAL	
		Cases	Ave. pH	Cases	Ave. pH	Cases	Ave. pH
3 weeks to 1 year	9 A.M.	11	6.73	8	6.87	19	6.79
	10 "	11	6.87	18	6.93	29	6.90
	11 "	12	6.74	10	6.70	22	6.72
	Total	34	6.78	36	6.85	70	6.81
	1 P.M.	6	6.78	7	6.75	13	6.77
	2 "	5	7.02	8	6.83	13	6.90
	3 "	4	6.70	2	7.20	6	6.86
	4 "	2	7.05	0	—	2	7.05
	Total	17	6.86	17	6.84	34	6.85
	Totals.....	51	6.81	53	6.85	104	6.83
1 to 6 years	9 A.M.	29	6.71	32	6.74	61	6.72
	10 "	28	6.75	30	6.72	58	6.73
	11 "	3	6.86	12	6.73	15	6.76
	Total	60	6.73	74	6.73	134	6.73
	1 P.M.	31	6.74	35	6.73	66	6.73
	2 "	7	6.91	14	6.90	21	6.90
	3 "	17	6.87	3	6.70	20	6.85
	Total	55	6.80	52	6.77	107	6.79
	Totals.....	115	6.76	126	6.75	241	6.75
6 to 10 years	9 A.M.	38	6.81	47	6.75	85	6.77
	10 "	23	6.85	32	6.77	55	6.80
	11 "	28	6.80	19	6.83	47	6.81
	Total	89	6.82	98	6.76	187	6.79
	1 P.M.	19	6.70	23	6.85	42	6.78
	2 "	14	6.89	24	6.79	38	6.82
	3 "	0	—	13	6.86	13	6.86
	Total	33	6.77	60	6.82	93	6.80
	Totals.....	122	6.81	158	6.79	280	6.80
10 to 20 years	8 A.M.	26	6.79	0	—	26	6.79
	9 "	124	6.75	58	6.67	182	6.72
	10 "	119	6.75	114	6.76	233	6.75
	11 "	76	6.82	122	6.76	198	6.78
	Total	345	6.77	294	6.74	639	6.76
	12 Noon	40	6.87	83	6.80	123	6.82
	1 P.M.	57	6.65	42	6.58	99	6.62
	2 "	38	6.73	47	6.67	85	6.71
	3 "	9	6.82	13	6.75	22	6.77
	Total	144	6.74	185	6.72	329	6.73
	Totals.....	489	6.76	479	6.73	969	6.74
20 to 30 years	8 A.M.	1	7.20	31	6.75	32	6.76
	9 "	16	6.85	25	6.79	41	6.81
	10 "	13	6.83	40	6.83	53	6.83
	11 "	18	6.80	23	6.70	41	6.74
	Total	48	6.83	119	6.77	167	6.79
	12 Noon	1	6.90	1	7.10	2	7.00
	1 P.M.	41	6.73	12	6.73	53	6.73
	2 "	59	6.76	13	6.77	72	6.76
	3 "	21	6.90	26	6.74	47	6.81
	4 "	0	—	14	6.71	14	6.71
	Total	122	6.77	66	6.76	188	6.77
	Totals.....	170	6.79	185	6.77	355	6.78
30 to 40 years	7 A.M.	6	6.76	0	—	6	6.76
	8 "	9	6.56	12	6.69	21	6.64
	9 "	2	6.80	15	6.74	17	6.75
	10 "	7	6.73	17	6.67	24	6.69
	11 "	8	6.95	13	6.67	21	6.77
	Total	32	6.75	57	6.70	89	6.72
	12 Noon	2	6.70	0	—	2	6.70
	1 P.M.	42	6.64	12	6.79	54	6.67
	2 "	38	6.74	16	6.81	54	6.76
	3 "	9	6.54	27	6.60	36	6.59
	4 "	4	6.40	1	6.70	5	6.44
	Total	95	6.66	56	6.70	151	6.67
	Totals.....	127	6.69	113	6.70	240	6.69

TABLE 1—*Continued*

AGE-GROUP	HOUR	MALE		FEMALE		TOTAL	
		Cases	Ave. pH	Cases	Ave. pH	Cases	Ave. pH
40 to 50 years	7 A.M.	4	6.80	0	—	4	6.80
	8 "	21	6.73	0	—	21	6.73
	9 "	6	6.60	14	6.83	20	6.76
	10 "	13	6.67	18	6.69	31	6.68
	11 "	22	6.84	16	6.80	38	6.82
	Total	66	6.74	48	6.77	114	6.75
	1 P.M.	20	6.52	28	6.75	48	6.66
	2 "	50	6.78	18	6.85	68	6.80
	3 "	27	6.73	21	6.84	48	6.78
	4 "	8	6.87	0	—	8	6.87
	Total	105	6.73	67	6.80	172	6.76
	Totals.....	171	6.73	115	6.79	286	6.76
50 to 60 years	7 A.M.	26	6.90	0	—	26	6.90
	8 "	35	6.68	0	—	35	6.68
	9 "	4	7.02	0	—	4	7.02
	10 "	25	6.78	19	6.67	44	6.73
	11 "	33	6.81	15	6.68	48	6.76
	Total	123	6.79	34	6.67	157	6.77
	1 P.M.	45	6.67	30	6.66	75	6.67
	2 "	47	6.78	17	6.71	64	6.76
	3 "	40	6.76	14	6.63	54	6.73
	4 "	8	6.86	15	6.69	23	6.74
	Total	140	6.75	76	6.67	216	6.72
	Totals.....	263	6.77	110	6.67	373	6.74
60 to 70 years	7 A.M.	20	6.92	0	—	20	6.92
	8 "	26	6.80	0	—	26	6.80
	9 "	3	6.80	0	—	3	6.80
	10 "	11	6.88	11	6.59	22	6.73
	11 "	23	6.72	5	6.92	28	6.75
	Total	83	6.82	16	6.69	99	6.79
	1 P.M.	41	6.65	20	6.67	61	6.66
	2 "	36	6.78	7	6.81	43	6.78
	3 "	21	6.84	11	6.80	32	6.83
	4 "	7	6.49	6	6.86	13	6.66
	Total	105	6.72	44	6.75	149	6.73
	Totals.....	188	6.76	60	6.74	248	6.76
70 to 80 years	7 A.M.	4	6.82	0	—	4	6.82
	8 "	8	6.70	0	—	8	6.70
	9 "	0	—	0	—	0	—
	10 "	1	6.80	16	6.60	17	6.61
	11 "	5	6.54	14	6.65	19	6.62
	Total	18	6.69	30	6.63	48	6.65
	1 P.M.	6	7.05	15	6.60	21	6.73
	2 "	16	6.78	16	6.72	32	6.75
	3 "	31	6.65	22	6.60	53	6.63
	4 "	38	6.76	26	6.77	59	6.76
	Total	86	6.75	79	6.68	165	6.71
	Totals.....	104	6.73	109	6.66	213	6.70
80 to 90 years	10 A.M.	1	6.40	8	6.45	9	6.44
	11 "	0	—	6	6.80	6	6.80
	Total	1	6.40	14	6.60	15	6.59
	1 P.M.	0	—	14	6.57	14	6.57
	2 "	1	6.40	10	6.44	11	6.43
	3 "	11	6.91	10	6.53	21	6.73
	4 "	12	6.90	15	6.66	27	6.77
	Total	24	6.88	49	6.56	73	6.67
	Totals.....	25	6.86	63	6.57	88	6.63
90 to 100 years	10 A.M.	0	—	1	6.70	1	6.70
	11 "	0	—	1	6.60	1	6.60
	Total	0	—	2	6.65	2	6.65
	1 P.M.	0	—	3	6.63	3	6.63
	2 "	1	6.60	1	6.40	2	6.50
	3 "	0	—	0	—	0	—
	4 "	0	—	1	6.50	1	6.50
	Total	1	6.60	5	6.56	6	6.58
	Totals.....	1	6.60	7	6.60	8	6.60

Results. *Table 1* shows the average pH of normal resting saliva of different age-groups, both males and females, at various hours. *Table 2* is a composite showing average pH at various hours for the entire group. *Table 3* gives the frequency distribution at various hours for males, females, and total males and females. *Fig. 1* shows curves of the average at various hours for males, females, and total males and females.

Discussion. The average pH (6.88, *table 2*) for salivas collected from 7 to 8 A.M. is considerably higher than that (6.75) for all determinations. The former determinations were made on saliva collected one to two hours after rising and before breakfast. The average values for the 8, 9, 10, and 11 o'clock groups do not vary appreciably from the average for the entire group. The determinations for the 12 o'clock group, made before lunch, are higher—average, 6.83. The average, 6.68, for the 1 o'clock group, for saliva obtained 30 minutes after the noon meal, is considerably below that for the 12 o'clock group, and appreciably below that of the average for the entire group. The averages for the 2, 3, and 4 o'clock-groups are very similar, and

TABLE 2

Average pH of normal resting saliva of males and females of different ages, from 7:00 to 11:00 A.M., and from 12 to 5:00 P.M.

HOUR	MALE		FEMALE		TOTAL	
	Cases	Ave. pH	Cases	Ave. pH	Cases	Ave. pH
7 A.M.	60	6.88	—	—	60	6.88
8 A.M.	126	6.73	43	6.73	169	6.73
9 A.M.	233	6.76	199	6.74	432	6.75
10 A.M.	252	6.77	324	6.74	576	6.75
11 A.M.	228	6.79	256	6.75	484	6.77
Total	899	6.78	822	6.74	1721	6.76
12 Noon	43	6.86	84	6.81	127	6.83
1 P.M.	308	6.67	241	6.69	549	6.68
2 P.M.	312	6.77	191	6.75	503	6.76
3 P.M.	190	6.77	162	6.71	352	6.74
4 P.M.	74	6.77	78	6.73	152	6.75
Total	927	6.75	756	6.73	1683	6.74
Totals.....	1826	6.76	1578	6.73	3404	6.75

TABLE 3

Frequency distribution of the pH of normal resting saliva, at various hours, of males, females, and total males and females.
(M = males; F = females; T = total males and females)

pH		TIME OF DAY																						TOTAL		
		7–8			8–9			9–10			10–11			11–12			12–1			1–2			2–3			3–4			4–5					
		M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T	M	F	T
5.6–5.8	M	0			0			0			0			0			0			0			0			0			0			0		
	F		0			0			0			0			0			0			0			1			1			0			2	
	T			0			0			0			0			0			0			1			1			0			2	
5.8–6.0	M	1			1			1			0			1			0			4			1			0			1			10		
	F		0			0			2			2			0			0			3			2			4			0			13	
	T			1			1			3			2			1			0			7			3			4			1			23
6.0–6.2	M	0			8			6			4			3			0			9			4			4			2			40		
	F		0			0			1			9			4			1			5			1			6			3			30	
	T			0			8			7			13			7			1			14			5			10			5			70
6.2–6.4	M	1			6			5			1			11			1			35			20			7			7			94		
	F		0			5			11			10			8			1			11			8			8			3			65	
	T			1			11			16			11			19			2			46			28			15			10			159
6.4–6.6	M	1			14			25			26			24			1			50			33			28			11			213		
	F		0			4			26			46			37			2			42			29			24			11			221	
	T			1			18			51			72			61			3			92			62			52			22			434
6.6–6.8	M	14			28			68			62			51			7			67			73			47			12			428		
	F		0			11			54			78			75			25			69			44			49			18			424	
	T			14			39			122			140			126			32			136			117			96			30			852
6.8–7.0	M	16			32			68			97			63			17			81			91			55			18			540		
	F		0			14			61			98			67			32			66			58			30			31			467	
	T			16			46			129			197			140			49			147			149			85			49			1007
7.0–7.2	M	15			25			40			41			53			15			36			47			30			8			313		
	F		0			9			32			58			35			19			35			29			20			6			242	
	T			15			34			72			99			88			34			71			76			52			14			555
7.2–7.4	M	9			8			20			17			16			2			24			35			9			9			149		
	F		0			0			12			21			19			3			7			13			11			6			92	
	T			9			8			32			38			35			5			31			48			20			15			241
7.4–7.6	M	2			3			0			1			5			0			1			6			7			4			29		
	F		0			0			0			1			1			1			3			6			9			0			21	
	T			2			3			0			2			6			1			4			12			16			4			50
7.6–7.8	M	1			1			0			1			1			0			1			2			1			2			10		
	F		0			0			0			1			0			0			0			0			0			0			1		
	T			1			1			0			2			1			0			1			2			1			2			11
Totals	M	60			126			233			252			228			43			308			312			190			74			1826		
	F		0			43			199			324			256			84			241			191			162			78			1574	
	T			60			169			432			576			484			127			549			503			352			152			3404

do not vary appreciably from the general average. In general, the data show that the average pH of normal resting saliva rises before meals and drops after meals. After breakfast it drops to the normal level; after lunch, below the normal level. The normal level is maintained for approximately three hours between meals.

The data in *table 1* indicate that the averages for pH of normal resting saliva of the different age-groups vary throughout the day like

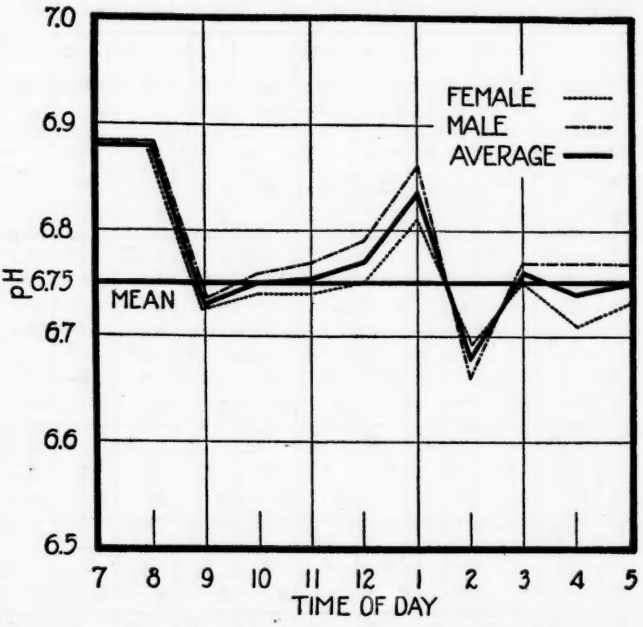

FIG. 1. GRAPH SHOWING AVERAGE pH OF SALIVA AT VARIOUS HOURS FOR MALES, FEMALES, AND TOTAL MALES AND FEMALES

those for the entire group. The curves in *fig. 1* show that the averages for males and females vary similarly throughout the day. The results for diurnal variations agree, in general, with those of Henderson and Millet (6), who noted that salivary pH dropped after each meal to a point slightly below that of the previous meal, and then rose gradually between meals. The work of Starr (11), who found the saliva more acid after meals, is also confirmed. The data do not agree with those

of Bloomfield and Huck (1), who stated that diurnal variation is inconstant and the same as the individual variations. They made only four determinations, at times when the diurnal variations reported in this paper would not have been in evidence.

At present there seems to be considerable disagreement in the literature concerning an explanation for the change in salivary pH. Broderick (3) believes it is due to a change in the H_2CO_3:$BHCO_3$ ratio of the blood. This view is supported by Starr (11), who observed that pH varied directly with the alveolar CO_2. Champion (4) and Smith (10) found no evidence to support the theory that the reaction of saliva varies with that of the blood. It is highly desirable that more work should be done on this phase of the subject.

The author's data differ from those of La Farga (7), who found saliva very acid in the morning and alkaline in the afternoon. The data in *table 1* show that no significant difference exists between the averages for resting saliva in the morning (6.76) and afternoon (6.74). Inasmuch as the pH range, 5.6 to 7.6, of normal resting saliva is the same for morning and afternoon, it is quite possible that one might obtain acid or alkaline values in either period, especially if only a few determinations were made. The value obtained for the case 101 years of age, not included in the tables, was pH 6.20, taken at 4:00 P.M.

Summary and conclusions. 1. Colorimetric pH determinations were made in normal resting salivas of 3405 normal healthy individuals, both male and female, ranging in age from 3 weeks to 101 years. Determinations were made at various hours of the day from 7 A.M. to 5 P.M.

2. The average pH varies during the day. During the hour before meals the average is slightly higher than the normal (6.75). It was 6.8 the hour before breakfast; 6.83 the hour before lunch. The average is slightly lower than the normal level during the hour following meals. It was 6.73 after breakfast; 6.68 after lunch.

3. The diurnal variations for the different age-groups are similar.

4. The variations in the average throughout the day, for males and females, are similar.

5. There is no significant difference between the average in the morning (pH 6.76) and in the afternoon (pH 6.74).

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STUDIES OF THE pH OF NORMAL RESTING SALIVA. III. EFFECTS OF VITAMINS A AND D IN SCHOOL CHILDREN[1]

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The pH of saliva has been studied in relation to dental caries by both environmental and nutritional schools. Hawkins[2] concluded that salivary pH is related to the occurrence of caries, pyorrhea, and chronic infections. To neutralize mouth acidity for the control of dental caries, he suggested (as one factor) maintenance of high salivary alkalinity, his dietary measures for this purpose including such foods as fruits, vegetables, milk, and nuts, with low proportions of cereal. He stated that meat, fish, cheese, and vitamin D *increase* the acidity of saliva; Forbes and Gurley[3] concluded that a high-cereal or a high-grain diet tends to *decrease* its acid-neutralizing power, a high-meat, high-egg, high-vegetable and fruit diet *increasing* its acid-neutralizing power. (The pH of saliva was used by Hawkins and by Forbes and Gurley as an indication of the acid-neutralizing power.) Hanke,[4] using a standard diet augmented by the juice of one lemon and sixteen ounces of orange juice (sources of vitamin C) for a period of one year, found no appreciable change in pH of various regions of the mouth. The object of this paper is to record the effects of administration of vitamins A and D upon the pH of normal resting saliva. This study was made in connection with an earlier one by Day and Sedwick.[5]

Procedure. The method (colorimetric) was outlined in a previous paper.[6] School children (133), ranging in age from 11 to 17 years (average, 13), were each given six vitamin-concentrate tablets daily

[1] Presented at the thirteenth general meeting of the International Association for Dental Research, Chicago, March 16 and 17, 1935.

[2] Hawkins: *J. Amer. Den. Assoc.*, 1929, *16*, 781; *J. Den. Res.*, 1931, *11*, 201; *J. Amer. Den. Assoc.*, 1932, *19*, 963. [3] Forbes and Gurley: *J. Den. Res.*, 1932, *12*, 637. [4] Hanke: Diet and dental health, 1933, 112; Chicago. [5] Day and Sedwick: *J. Den. Res.*, 1934, *14*, 214. [6] Brawley, R. E.: *Ibid.*, 1935, *15*, 55.

for one year. Each tablet contained at least 1500 units of vitamin A and 2450 units of vitamin D. Another group of 164 school children of similar age was used as a control. The pH of normal resting saliva was determined, for both groups, at the end of six and twelve months.

Results and discussion. The results are summarized below:

Average pH of normal resting saliva of experimental and control groups at end of six and twelve months

	Experimental		*Control*	
	Cases	*Ave. pH*	*Cases*	*Ave. pH*
End of six months.....	133	6.76	164	6.75
End of twelve months.....	133	6.79	164	6.78

There was no significant difference between the average pH of normal resting saliva of the children in the experimental group and that of the control group at the end of either six or twelve months. There was no significant difference between the averages after six months and twelve months. These averages are not appreciably different from that (6.74) of normal resting saliva for age-group 10–20 years, previously reported.[6]

These results indicate that vitamins A and D, when administered simultaneously in a concentrate-tablet for a period of one year, do not significantly affect the pH of saliva. Hanke obtained similar results after supplementing the diet for one year with vitamin C (in lemon and orange juice). It thus appears that any change in salivary pH induced by dietary measures cannot be attributed to vitamins alone. The pH changes reported by Hawkins and by Forbes and Gurley may have depended on the ash reaction of the diet, or on a combination of this factor with vitamins.

Summary and conclusions. 1. Colorimetric pH determinations, on normal resting saliva of school children, to determine effects of daily administration of concentrate-tablets of vitamins A and D, were made at the end of six months and twelve months.

2. The average pH at the end of six-months was: experimental, 6.76; control, 6.75—at the end of twelve-months: experimental, 6.79; control, 6.78.

3. There was no significant difference between averages for those receiving the tablets and the controls, at the end of either the six- or twelve-month period.

THE IRON CONTENT OF TEETH OF NORMAL AND ANEMIC RATS

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In studies in this laboratory, the presence of iron in keratin of human dental enamel was demonstrated qualitatively by Rosebury;[2] and, with the aid of a micro-method involving color comparison of the very slightly dissociated red dipyridyl ferrous complex, Engel[3] found that the quantity of iron in human dental enamel is about 0.001 percent. It was of interest therefore to extend these investigations to pathological conditions whose variations may possibly elucidate the functional rôle of iron in dentition. Kitchin and McFarland[4] observed that the enamel on the labial aspect of incisors in rats subjected to nutritional anemia is much lighter in color than that of normal animals. This observation suggests that dietary insufficiency, and consequent depletion in the body-store of iron, may be responsible for this loss of color. The present paper is concerned with a quantitative comparison of the iron content of the teeth of anemic and normal rats.

Fourteen albino rats, all litter-mates, were separated, when 33 days old, into a control group and an experimental group. The controls received "Full-O-Pep Egg Mash" ad lib, an entirely adequate diet. The experimental group was given pasteurized milk ad lib, each rat also receiving 25 gm. of carrots per week; this group was housed in cages that were heavily galvanized and equipped with screen bottoms—conditions that reduced the amount of available extraneous iron. The growth curves duplicated the differences found by Kitchin

[1] William J. Gies Fellow in Biological Chemistry at Columbia University, 1934–35.

[2] Rosebury: Presence of iron in enamel keratin, *J. Den. Res.*, 1934, *14*, 269.

[3] Engel: Method for the determination of iron in dental enamel, *Ibid.*, 1934, *14*, 273.

[4] Kitchin and McFarland: Does pasteurized milk cause variation from normal in the crystal structure of enamel formed during the period of use of such milk?, *Ibid.*, 1933, *13*, 359.

and McFarland under similar conditions. *At the end of the experiment*, the *control* males weighed 228 to 262 grams; the females, 156 to 173 grams—the *anemic* males, 140 to 182 grams; the females, 123 to 128 grams. After the rats had been on the diet 66 days, the color of the labial enamel in the experimental group was lighter than that of the controls. At this time also the rats of the experimental group presented the gross physical appearance of anemia—pale eyes, ears, and toes—which became accentuated during the remainder of the experimental period. After being on the diet for 88 days, determinations of hemoglobin were made by the Sahli method. The results indicate that the hemoglobin content of the blood of the experimental animals was reduced to about half the normal amount (*table 1*):

TABLE 1

Hemoglobin content of blood of normal and anemic rats

CONTROL GROUP			ANEMIC GROUP		
Rat	Sex	Hemoglobin	Rat	Sex	Hemoglobin
no.		*percent*	no.		*percent*
13	M	96.0	9	M	48.5
5	M	96.5	10	M	—
12	M	97.5	11	M	53.0
14	M	91.0	6	M	48.5
4	F	88.0	8	M	48.5
2	F	90.5	7	M	46.5
			3	F	47.5
			1	F	46.5

After the experiment had continued 124 days, the animals were chloroformed. The whole incisors were cautiously dissected out by a technique that was made as uniform as possible; the outside of each tooth was carefully cleaned with cotton-covered probes moistened with distilled water; and the teeth dried overnight at 100°C. The color differences previously observed persisted after drying: the labial enamel of the controls had a deep orange color, all of approximately the same intensity, while that of the anemic group varied from a yellow-orange to practically no color. Since the pulp of a tooth contains some iron in the form of hemoglobin, it was essential, of course, to aim to eliminate this additional variable, but the minuteness of the canal-bore at the occlusal end makes it impossible to remove the

pulp completely by any simple physical means that avoids contamination with iron. A few preliminary experiments were conducted to investigate the removal of hemoglobin-iron, as its intact complex, by extracting with a reagent that would leave unaltered any other iron present. Heating fragments of a tooth for three hours with $N/10$ NaOH was found to be completely satisfactory for this purpose. Added hemoglobin is completely extracted from tri-calcium phosphate by this procedure, the original iron content of the sample remaining unchanged.

The two upper incisors of each rat were used for analysis. One in each rat was analyzed whole, without any preliminary treatment

TABLE 2

Iron content of upper incisors of normal and anemic rats

CONTROL GROUP			ANEMIC GROUP		
Rat	Untreated	Alkali treated	Rat	Untreated	Alkali treated
no.	*percent*	*percent*	*no.*	*percent*	*percent*
12	0.0280	.....*	11	0.0251	0.0218
4	.0308	.....*	10	.0208	.0160
13	.....*	0.0266	7	.0183	.0154
14	.....*	0.0266	1	.0119	.0112
5	.0297	.0259	6	.0120	.0111
2	.0305	.0255	9	.0105	.0100
			8	.0082	.0080
			3	.0086	.0075
Average	*0.0298*	*0.0262*	*Average*	*0.0144*	*0.0126*

* Lost by accident.

("untreated"). The other was broken into fragments in an agate mortar so that all canal surfaces were exposed; transferred to a small centrifuge tube (about 3 cc. in capacity); and there treated with 1 cc. of $N/10$ NaOH. The tube was loosely covered with a glass cap and heated in a water bath at 80–90°C. for three hours; then centrifuged; the supernatant liquid pipetted off; and the contents washed three times with 0.3-cc. portions of $N/10$ NaOH and once with 1 cc. of water, heating a few minutes—centrifuging each time before removing the wash liquid—and finally dried at 100°C. overnight. The dry fragments were then transferred to a tared crucible and weighed for analysis. The color of the labial enamel was not altered by this

procedure. In all instances the determinations were made by the micro-method described in detail by Engel, in which a few minor changes have been made. For the ashing, 2 cc. of conc. HNO_3 were found to be necessary. After dissolving the ash, when transferring to volumetric flasks, two 0.25-cc. portions of $N/10$ HCl were used to rinse the crucible, followed as usual by 2–3 cc. of water in four or five portions. From 3.0 to 3.5 cc. of $N/10$ NaOH were required to bring the solution to the proper pH. The method was standardized by means of a solution of pure iron wire in dilute HCl. The analytical results are summarized in *table 2*.

The average iron content of the teeth of the anemic animals was about half that of the controls; considerably more variation occurred in the experimental group. Although a strict correlation between color depth and iron content was not established, because the color of the enamel can be only crudely estimated, a general correlation does exist, and the conclusion is warranted that iron is associated with the color. The question whether the iron is present *chiefly* as a part of the enamel pigment, or is otherwise distributed in the tooth structure, cannot be decided from this preliminary study.

It is of interest to note that much more iron is present in rat incisors than in human dental enamel. If the comparison is valid, it would seem to support the conclusions presented above, since human dental enamel is almost devoid of color.

The author wishes to acknowledge her indebtedness to Professors Wm. J. Gies and H. T. Clarke for their generous interest and advice.

MEDICO-DENTAL CASE RECORDS: XII. FUSOSPIROCHETAL ANGINA

A Clinico-pathological Study[1]

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I. Introduction. The case records in this series of reports, on clinical manifestations of the relation of oral sepsis to systemic disease, originally included only cases in which the secondary lesion was the result of a *metastatic* infection arising from a primary chronic periapical or periodontal oral focus. As the study proceeded however, it became evident that there was an important group of cases which should be included in the study and yet which were not within the above classification. This group included cases in which the secondary lesion was the result of *direct extension* of a primary chronic oral focus of infection. Cases in which the secondary lesion is the result of direct extension of acute infections *per se* constitute a third group, which are not considered in the present series. The sixth report[1] was the first in this series to describe a secondary lesion (noma) resulting from direct extension of a primary chronic focus (fusospirochetal infection). The present case record is the second in this series to describe a case in which the fatal secondary lesion resulted from direct extension of a primary chronic periodontal fusospirochetal infection.

The number of reported fatal cases of fusospirochetal angina has been relatively small, although it is generally believed that fatalities are not so rare as reports of cases might indicate. Goldman and Kully[2] recently reported 7 cases (all adult Negroes) of fatal fusospiro-

[1] Previous reports were published in the *Journal of Dental Research*: (1) 1926, vi, p. 283; (2–4) 1927, vii, pp. 115, 275, 457; (5–6) 1928, viii, pp. 73, 579; (7) 1929, ix, p. 89; (8–9) 1930, x, pp. 173, 675; (10) 1931, xi, p. 847; (11) 1932, xii, p. 713. Presented at the thirteenth general meeting of the International Association for Dental Research, Chicago, March 16 and 17, 1935.

[2] Goldman and Kully: Fatal fusospirochetal angina, *J. Amer. Med. Assoc.*, 1933, ci, p. 358.

chetal angina among 21 patients (52 percent Negroes) admitted to the Cincinnati General Hospital during 1929–1932 with diagnosis of fusospirochetal angina. These authors refer also to other reports of fatal fusospirochetal angina. In 1904, Bruce[3] discussed a fatal case in a girl, aged 8, in which the ulcerations extended throughout fauces, base of tongue and epiglottis; and another case in a boy, aged 9, who died after a prolonged illness of thirty-seven days with ulcerations extending to the thyroid cartilage and with multiple lung abscesses. Halstead[4] alluded to two fatal cases, and cited others of Di Corlis, Gilberti, and Meyer. Heinemann,[5] in 1917, described a large series of fatal cases, occurring in an epidemic in Adrianople, the fusospirochetal angina extending to noma. Fraenkel's[6] cases in 1922 presented extensive ulceration and bronchopneumonia. Husik[7] published a case that was fatal during a secondary anemia, the ulcerations extending to the upper part of the bronchial tree.

II. CASE S-4: FUSOSPIROCHETAL ANGINA. GENERAL HISTORY. *Patient:* I. O., white, female, 55 years; admitted to hospital[8] late at night July 31; first seen in consultation following morning. *Chief complaint:* painful and difficult swallowing; swelling and pain, right side of face; general malaise. *Family history:* mother died of cerebral hemorrhage; father, of pneumonia; four sisters living and well. *Past medical history:* had common childhood diseases, otherwise always in good health. *Respiratory system:* no pain in chest; no cough. Moderate dyspnoea since onset of present acute condition. *Cardio-vascular system:* swelling of feet and ankles for several months; dyspnoea and palpitation on exertion, no hemoptysis, hematemesis, or epistaxis; no epigastric or precordial pain.

[3] Bruce: Vincent's angina, *Lancet*, 1904, ii, p. 135.

[4] Halstead: Vincent's angina: its frequency and the importance of its recognition, *Laryngoscope*, 1912, xxii, p. 1372.

[5] Heinemann: Beobachtungen über Stomatitis ulcerosa, Angina Vincent, und Noma, *Berl. Klin. Wchnschr.*, 1919, no. 5; *Derm. Centr.*, 1919, p. 152.

[6] Fraenkel: Ueber Plaut-Vincentsche Angina, *Ztschr. f. Hyg. u. Infectionskr.*, 1922, xcvii, p. 162; *Centr. f. Haut-u. Geschlechtskr.*, 1922, vii, p. 495.

[7] Husik, D. N.: A fatal case of Vincent's disease, *Ann. Otol., Rhin. and Laryng.*, 1922, xxxi, p. 1039.

[8] Admitted to the medical service, Fifth Avenue Hospital, New York City, by Joseph Lintz, M.D., and referred for consultation and surgical treatment to Charles Lester, M.D., Department of Surgery, and Malcolm W. Carr, D.D.S., Department of Oral Surgery. The authors are indebted to Drs. Lintz and Lester for coöperation in the publication of this report.

Gastro-intestinal system: no diarrhea; no constipation; no nausea or vomiting; no pain in abdomen; no congestion of superficial veins. Appetite poor; past six months, food limited principally to fluids—poorly balanced; loss of weight. *Genito-urinary system:* nocturia 3–4x; diuria 4–5x; no dysuria, hematuria, polyuria, or incontinence. *History of present illness:* six months ago had "bilious attack," diagnosed as gall-bladder condition; followed by recurrent attacks of stomatitis, diagnosed as Vincent infection and treated by intravenous injections of salvarsan. Three months ago "double abscess developed which was incised." Three weeks ago had another recurrent attack of Vincent's stomatitis, focal points of which were third molar regions. Infection yielded to local treatment by dentist except in region of right mandibular third molar, where it was difficult to control and persisted in subacute phase. Ten days ago right mandibular third molar removed; procaine anesthesia. Moderate swelling followed removal of tooth, soreness continued; "discharge from socket, which continued until 24 hours before admission, when discharge ceased and simultaneously face and neck on right side became extensively swollen." In last few hours swallowing has become difficult.

PHYSICAL EXAMINATION. *General appearance:* well-developed but poorly nourished; evidence of loss of weight; moderate pallor; no cyanosis; extensive swelling of right facial and submaxillary tissues; speech blurred; moderate dyspnoea; respiration principally through mouth; extreme nervousness and great anxiety over condition. General appearance of one acutely and seriously ill. Temperature, 103.8; pulse, 100; respiration, 24. *Head:* no deformities or irregularities, exostoses or cranial deformities. *Scalp:* no scars. *Eyes:* no paralysis of ocular musculature; no strabismus, nystagmus, ptosis nor exophthalmos; no evidence of inflammation or infection; pupils react to light and accommodation. *Ears:* no discharge; no mastoid tenderness or swelling. *Nose:* mucosa congested and hyperemic; no obstruction or discharge. *Maxillo-facial region; external appearance:* extensive and diffuse cellulitis, with massive induration of facial and submaxillary tissues extending from malar and zygomatic processes above, to level of thyroid cartilage below, and from sternocleidomastoid muscle to symphysis, with infiltrating edema extending across median line to opposite side of neck; anterior and posterior cervical adenopathy. Marked tenderness throughout region of swelling; redness not pronounced, no fluctuation or pitting on pressure. *Intra-oral examination:* trismus moderate, possible to open mouth 2.5 cm.; pronounced induration of buccal tissues, approximately 2.5 cm. in thickness and bulging over occlusal surfaces of mandibular teeth; buccal mucosa furrowed and somewhat dis-

colored, with no indication of fluctuation. Extensive area of sloughing extending from socket of right mandibular third molar on lingual side, continuous with floor of mouth, involving side of tongue and extending toward base of tongue. Lingual plate of mandible exposed from region of mandibular second molar to the angle. Slough also extended along anterior faucial pillar, involving soft palate to median line. Floor of mouth not elevated (as in Ludwig's angina); moderate edema and only slight induration of anterior portion of floor. Tongue swollen, edematous, with blackish discoloration, suggesting superficial necrosis. *Fetor ex ore* marked. Periodontal disease not pronounced elsewhere in mouth; moderate chronic gingivitis not typical of Vincent stomatitis. Teeth moderately well cared for. *Roentgenographic examination:* right mandible—no evidence of necrosis, osteomyelitis or periosteal change. *Chest:* contour normal; expansion normal on both sides; no areas of tenderness or swelling. *Lungs:* no change in percussion note; no abnormalities in vocal or tactile fremitus. On auscultation, occasional dry râle heard near right base; otherwise chest clear. *Heart:* apex beat in fifth interspace in mid-clavicular line; no thrill; no murmurs; no arrhythmias; second aortic sound accentuated. *Blood pressure:* systolic 220 mm.; diastolic 115 mm. *Abdomen:* markedly distended and moderately tense; no tenderness or rigidity; no palpable masses; presence of shifting dullness in flanks, and suggestion of fluid wave. *Genitalia:* negative. *Extremities:* feet, ankles, legs, thighs, sacrum and lumbar region pit on pressure; no discoloration of skin; no ankylosis of joints or abnormal movements; no evidence of varicosities of veins or sclerosis of arteries.

CLINICAL PATHOLOGY. *Urinalysis:* specific gravity, 1.008; reaction, alkaline; albumin, ++++; sugar, trace; moderate number of epithelial cells and white blood-cells. *Hematology:* hemoglobin, 62 percent; red blood-cells, 3,000,000; white blood-cells, 18,900; polymorphonuclear leukocytes, 82 percent; lymphocytes, 18 percent; coagulation, time, 4 minutes. *Bacteriology:* smear from gangrenous area positive for fusospirochetal infection, with large numbers of fusiform bacilli and spirochetes.

DIAGNOSIS AND RECOMMENDATIONS. *Clinical diagnosis:* fusospirochetal angina, gangrenous stomatitis, diffuse facial and submaxillary cellulitis, general toxemia, moderate loss of weight (from food insufficiency), secondary anemia and hypertension. *Prognosis:* unfavorable. *Recommendations:* immediate operation to consist of external (submaxillary) incision for drainage and intra-oral incision for counter-drainage. Patient's serious condition justified immediate exploratory operation, to relieve pressure and tension.

TREATMENT AND PROGRESS NOTES. *Pre-operative treatment:* wet dressing of hot magnesium sulphate applied to face and neck; liquid diet, fluids forced (water ℥ viii every hour); 1000 cc. normal saline solution hypodermoclysis; morphine sulphate gr. ¼ given following admission. *Record of operation:* performed noon, Aug. 1, twelve hours following admission. *Pre-operative medication:* morphine sulphate gr. ¼. Skin just below angle of jaw infiltrated with 2 percent procaine and needle passed deeply to thoroughly infiltrate underlying tissue. Incision, about 2 cm. long through skin and superficial fascia parallel to lower border of mandible, about 1 cm. below it. Curved clamp inserted into incision and used to bluntly-explore tissues of neck until it could be felt beneath floor of mouth. Tissues well spread with clamp but no pus obtained. Fenestrated rubber tube inserted through incision, to base of tongue. Moderate hemorrhage controlled with gauze packing. Intraoral incision made in vestibule of mouth at reflection of mucoperiosteum and buccal mucosa, running from external oblique line of ramus anteriorly to region of second premolar. By blunt dissection tissues of cheek were explored but no pus found. Moderate hemorrhage controlled with iodoform gauze packing before patient left table. *Post-operative treatment and progress notes:* patient withstood operation moderately well. Wet dressings of hot magnesium sulphate continued. Mouth irrigated with hot sodium perborate every hour. At 3:30 P.M. bleeding from both incisions noted and controlled by packing with gauze. At 6:30 P.M. active bleeding again occurred; difficult to control by packing without use of anesthetic. General condition of patient had become worse since noon. Transfusion indicated and arrangements made for suitable donor, but immediate location of source of hemorrhage and effectual control of bleeding were imperative because of patient's rapidly declining condition and secondary anemia. Packing had been ineffectual as bleeding seemed to arise from deeper tissues, probably within area of extensive sloughing and owing to erosion of one of larger recesses. At 7:30 P.M. patient given avertin (80 mg.), preparatory for operation to control bleeding; condition became rapidly worse, and death occurred before operation could be performed. No autopsy. *Post-mortem diagnosis:* primary focal lesion; fusospirochetal gangrene; secondary or terminal lesions; secondary hemorrhage (from sloughing area in deep sublingual tissues, increased by hypertension), secondary anemia, toxemia, edema of glottis.

III. SUMMARY AND DISCUSSION. This patient had been suffering from recurrent attacks of Vincent stomatitis for six months. Although she had received local and constitutional treatments, the

infection continued to run a chronic course. Believing that the focal point was around the right mandibular third molar, this tooth was removed in an effort to eliminate the focus. Following the removal of the tooth, acute exacerbation occurred and persisted for ten days until the patient was first seen at the hospital, when extensive sloughing had already taken place; upon admission a direct smear showed great numbers of fusospirochetal organisms. Thus far the historical data relate to three local factors of importance that frequently influence the course of fusospirochetal infections of the mouth: local infection (chronic Vincent stomatitis), local trauma (removal of the tooth) followed by acute exacerbation, and perhaps a period of relative neglect during which extensive sloughing develops. In addition to these local factors, the patient presented constitutional factors which lowered her general resistance, thus favoring spread of local infection and development of advanced toxemia; she was poorly nourished from food insufficiency and an unbalanced diet, and suffered also from a moderate secondary anemia. When she was first seen all these factors were recognized, the prognosis being unfavorable principally because of the extensive sloughing (which favored increase in virulence of the organisms), the already high degree of toxemia, and the lowered general resistance of the patient. In addition to local sloughing, extensive facial and submaxillary cellulitis complicated the case, and operation for incision and drainage was performed to relieve pressure and tension. Sloughing of deep tissues was extensive, and moderate bleeding was encountered at the time of operation, principally from the deeper tissues of the floor of the mouth into which the slough had extended. Hypertension also probably contributed to the bleeding which, however, was controlled at the time of operation. Secondary hemorrhage occurred later, principally from the deeply sloughing areas, which could not be controlled by packing. The patient died eight hours after operation. Death was probably hastened by secondary hemorrhage and secondary anemia, although general toxemia and edema of the glottis, and possible metastatic lesions elsewhere, were the more important terminal lesions.

Plaut, Vincent, Muhlens, Kolle, Onorato, Beck, and others, have directed attention to the fact that fusiform bacilli and spirochetes are present in healthy mouths, where, however, they are not numerous.

In active cases of fusospirochetal infection, fusiform bacilli and spirochetes are present in great numbers—a quantitative difference from "normal." Furthermore, under certain conditions, these organisms may pass from a relatively saprophytic state to a definitely pathogenic state. These facts are mentioned by Goldman and Kully,[2] who state that the reason for this change is not clearly understood, but suggest that local infection (dental caries, periodontoclasia), *local trauma*, systemic infections, and intoxications may be influencing factors. The historical facts and clinical findings described in the foregoing case-report confirm this general concept. Pilot,[9] has aptly called fusiform bacilli and spirochetes "opportunists," which become pathogenic only when the integrity of the tissues holding them is altered. Goldman and Kully suggest also that uncommonly virulent strains of these organisms may exist, and that "when extensive ulcerations are provided in the mouth the other organisms present, especially the staphylococci and streptococci, play a very important rôle" in systemic invasion and involvement. The lesions caused by these organisms are of "great variety, depending to a large extent on the underlying factors, the resistance of the patient, and the behavior of the pathologic process. In all, the tendency to necrosis and the production of a foul-smelling lesion are striking characteristics."

IV. CONCLUSIONS. (1) Fusospirochetal angina is a potentially serious clinical entity. (2) Local factors such as neglect of chronic infection or of acute exacerbations, local trauma in the presence of chronic or acute infection, as well as secondary or associated constitutional factors, influence the clinical course of the disease. (3) When extensive ulceration and sloughing have occurred, the prognosis is invariably bad. (4) Death from fusospirochetal angina may be due to extension of the local pathologic process involving the glottis (edema), to toxemia or hemorrhage arising from extensive sloughing areas, or to occurrence of metastatic lesions elsewhere in the body.

[9] Pilot: Fusospirochetal infections of the mouth, *J. Amer. Den. Assoc.*, 1928, xv, p. 1763.

A CRITIQUE OF BOLK'S "PROBLEMS OF HUMAN DENTITION"

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Of the non-paleontological contributions to the knowledge of the origin of the human dentition the publications of the late Prof. Louis Bolk, the Dutch anatomist, are perhaps the most outstanding. His numerous articles are original and provocative. This critique is concerned with one of his chief papers: "Problems of Human Dentition" (2). It is not my aim to depreciate the significance of Bolk's observations. I am primarily concerned with the demonstration of certain fallacies and inconsistencies in the construction of his theory. He seems to have built a logic-tight system based upon two major premises: (a) so-called *reversions* repeat ancestral conditions, and (b) recapitulation of phylogeny in ontogeny. All of his arguments in anatomy and embryology are in some way dependent for their strength upon these two postulates, both of which, although popular with nineteenth century morphologists, are to-day seriously questioned by most geneticists, embryologists, and paleontologists. With the invalidation of the two premises, the theory collapses and "problems" of human dentition would remain problems. In the presentation of his work, Bolk began with an assumption concerning the taxonomic position of the marmosets. He then attempted to support the assumption by demonstrating its consistency with his theory of the transition from the Platyrrhinæ to the Catarrhinæ. According to this theory the marmosets are supposed to play an important rôle in the transition. The whole scheme, however, was patterned after a transition phenomenon in the recent human dentition, and pieced together from disconnected facts in comparative anatomy and embryology. The same recent phenomenon, after which his theoretical transition was modelled, was also used as a proof of that theory. It is somewhat difficult to analyse Bolk's method of reasoning because his material

was presented backwards. This in itself does not constitute a logical fallacy. His procedure, however, lacks demonstrative force because no independent evidence of the truth of the historical antecedent (other than its relation to the facts sought to be explained) is offered. Because he utterly disregarded the only source of such independent evidence, paleontology, his theory is unscientific. In order to prove his theory, it was necessary for Bolk to show actual fossil records substantiating his new phylogenetic arrangement. This he did not do. Bolk's reasoning was both unscientific and devoid of demonstrative force.

The *first problem* is entitled, "The relations between the dentition of Platyrrhine and Catarrhine Primates" (2, p. 92). Of the differences between the two large primate groups, the Platyrrhinæ (New World—more primitive) and the Catarrhinæ (Old World—more advanced), the most striking is that the former have three molars in their milk dentition and three premolars in their permanent dentition, while in the Catarrhinæ (which includes man) there are two molars in the milk dentition and two premolars in the permanent set. Bolk wrote the dental formulae for the two groups as follows (2, p. 93):

Platyrrhinæ: $i_1\ i_2\ c\ m_1\ m_2\ m_3$
$I_1\ I_2\ C\ P_1\ P_2\ P_3\ M_1\ M_2\ M_3$

Catarrhinæ: $i_1\ i_2\ c\ m_1\ m_2$
$I_1\ I_2\ C\ P_1\ P_2\ M_1\ M_2\ M_3$

Zoologists and paleontologists write these formulae a little differently:

Platyrrhinæ: $i_1\ i_2\ c\ m_2\ m_3\ m_4$
$I_1\ I_2\ C\ P_2\ P_3\ P_4\ M_1\ M_2\ M_3$

Catarrhinæ: $i_1\ i_2\ c\ m_3\ m_4$
$I_1\ I_2\ C\ P_3\ P_4\ M_1\ M_2\ M_3$

The latter method designates the premolars as 2, 3, and 4, indicating loss of P_1 which had existed in ancestral forms. Gregory ascribed to *Notharctus*, an American Eocene primate (7, p. 127), the dental formula: $I\frac{2}{2}C\frac{1}{1}P\frac{4}{4}M\frac{3}{3}$. *Necrolemur antiquus* had three upper and four lower premolars: $I\frac{2}{0}C\frac{1}{1}P\frac{3}{4}M\frac{3}{3}$ [Stehlin (12), p. 1329]. Bolk did not start with primitive mammalian or even primitive primate conditions, as would be the logical procedure in the treatment of this subject. Instead he began his arguments with the recent Platyrrhinæ. Bolk claimed that according to one of the popularly accepted theories,

the Catarrhinæ evolved from the Platyrrhinæ through loss of the first premolar and its predecessor, the first milk molar. Another theory maintains that the transition occurred through loss of the third premolar. Bolk disagreed with both. He argued that "in none or the recent genera of New World monkeys is a reduction of a tooth to be seen either of the first or third milk molar, or of the first or third premolar" (2, p. 93). His further objections to the popular theories were that "if really the first post-canine tooth in both dentitions should be reduced and lost, there is a very strong ground to expect that in the embryological evolution of the dentition of man, apes, or catarrhine monkeys, the anlage of this tooth or occasionally the tooth itself should be found in a rudimentary form and size. Without doubt the teeth are reduced and lost during the last phase of development in all mammals" (2, p. 94).

Bolk offered no grounds for expecting a lost tooth to reappear. He simply assumed that any character which may have been present in an ancestor, no matter how remote, will at some time reappear in descendants. As to reduction and loss of teeth in the "last phase of development in all mammals," Gregory (8, p. 284) points out that a premolar had already been lost in *Parapithecus* and *Propliopithecus* of the lower Oligocene some thirty million years ago. There are many other instances where the teeth were lost in an early phase of evolution. Bolk maintained that the catarrhine dentition evolved from the platyrrhine dentition in two steps (a) through loss of the third permanent molar, (b) the development of the third milk molar into a permanent tooth, "whilst the development of its successor (the third premolar) was suppressed, in consequence of which the number of the permanent molars increased to three, as in the primitive form" (2, p. 94). He claimed that the Hapalidæ (marmosets) show the first stage of the change, having lost their third permanent molars. From this fact Bolk concluded that the marmosets are on a higher level of evolutionary development than the Cebidæ, and added: "In most recent genera of New World monkeys—*Cebus*, *Ateles*, *Chrysothrix*, *Pithecia*, *Nyctipithecus*—the third molar is already reduced in a very large degree, having only a single root and a very small crown without cusp-differentiation" (2, p. 95). His concept of the evolution can be summarized by the following formulæ:

Cebidæ: $i_1\ i_2\ c\ m_1\ m_2\ m_3$
$I_1\ I_2\ C\ P_1\ P_2\ P_3\ M_1\ M_2\ M_3$
Hapalidæ: $i_1\ i_2\ c\ m_1\ m_2\ m_3$
$I_1\ I_2\ C\ P_1\ P_2\ P_3\ M_1\ M_2\ (M_3)$
Catarrhinæ: $i_1\ i_2\ c\ m_1\ m_2\ M_1$
$I_1\ I_2\ C\ P_1\ P_2\ (P_3)\ M_2\ M_3$

The suppressed elements are shown in parenthesis. Thus, according to Bolk, the first permanent molar of Catarrhinæ is homologous with the third milk molar of Platyrrhinæ. In *fig. 1* the theory is illustrated graphically. The following is a summary of Bolk's conception as to how the transition came about:

(*a*) "The evolution of the dental structure of Catarrhine primates commenced with the reduction and final loss of the hindmost molar . . . in consequence of the shortening of the jaws" (2, p. 97). (*b*) The grinding surfaces of the teeth were thus diminished. With the small marmosets, living on soft foods and insects, this made little difference. (*c*) But as the species "grew taller," the food required larger grinding areas. "It is very important that the third milk molar of the platyrrhine monkeys is a larger tooth, with a greater surface and more cusps than its successor, the third premolar" (2, p. 97). (*d*) "And so it was advantageous, to the grinding function of the dental arch of the historically succeeding larger forms of monkeys, that the third milk molar with its four or five cusps was not replaced by a tooth with two cusps only. Thus the grinding surface of the dental arch regained at its anterior end what it had lost in an earlier period of evolution at its posterior end" (2, p. 97).

There are some contradictions in Bolk's arguments. (*a*) His first statement concerning the beginning of the evolution of the dental structure of Catarrhinæ is simply an hypothesis. It was convenient for him at this moment to assume that the general tendency was toward reduction in size of the masticatory apparatus. He later assumed a tendency to increase in size. (*b*) He then arrived at the marmoset-stage of evolution where the animal is reduced to the size of a man's hand. He established the taxonomic position of the Hapalidæ on a single assumption concerning only one anatomical character. Gregory (8, p. 231)—after thorough consideration of such anatomical features as shape of skull, auditory meatus, eyes, vertical diameter of brain

case, maxilla, and nasal region—holds that the marmosets are a specialized rather than primitive form. Clark maintains that they "represent an off-shoot from the very base of the Platyrrhine stem" (4, p. 275). Gregory and Clark agree on the important feature of the taxonomic classification; namely, that marmosets are a side branch of Platyrrhinæ. They may disagree as to the time of the beginning of marmoset specialization, but neither regards Hapalidæ as a stage in the evolution of Catarrhinæ from Platyrrhinæ. As a matter of fact, the whole idea of the evolution of the Old World monkeys from New World monkeys is an assumption. Paleontological evidence indicates a common distant ancestry. (*c*) Bolk said that the species "grew taller" and therefore a larger grinding area was necessary. In his hypothetical phylogeny, jaws became shorter and species seemed to grow smaller or larger to meet the requirements of his theory. (*d*) The only explanations Bolk offered for the dental changes from one species to another are those in such phrases as "it is very important that the third milk molar . . . . is a larger tooth," and "it was advantageous" that the large tooth should not be replaced by a small one. Such arguments are not very persuasive.

Bolk attempted to support his theory by citing embryological and morphological affinities between the first permanent molar and second temporary molar of man. He said: "The anlage of milk teeth commences in the ninth week of embryological development. Immediately afterward the germ of the second milk molar is produced by the dental lamina, the latter is prolonged backward, and the enamel of the first permanent molar is formed. This happens in the sixteenth week of embryological development. Therefore, there is no discontinuity in the succession of the first anlage of the enamel-organs of our deciduous teeth and that of our first permanent molar. A relatively long time follows before the formation of the other teeth, especially the second permanent molar" (2, p. 98). These embryological relationships, however, do not prove that the present first permanent molar of man was originally the third deciduous molar. In 1916, Gregory (6, p. 243) suggested that the first permanent molar may be genetically of the same series as the temporary teeth, and cited evidence to support the view. But on additional grounds Bolk's embryological argument is untenable. He assumes that the order of

embryonic appearance of the teeth is an indication of the order of their phylogenetic appearance. This notion of recapitulation remains unsupported by most embryologists. The succession of events in ontogeny is no proof of evolutionary history. In embryological development, new features can appear earlier than ancient ones. Temporal and positional relationships in the developing organ do not reveal the past. "Evolutionary novelties," said deBeer (1, p. 40), "can and do appear in early stages of ontogeny." Thus Bolk's position is contradictory to both paleontological and embryological facts.

Bolk further attempted to support his stand by the morphological similarities between M_1 and m_2 in man. "As a rule," he said, "the form of cusp differentiation of M_1 shows more likeness to those of the second temporary molar than to those of the second permanent molar. . . . Our first permanent molar always seems to be a reproduction of the second milk molar on a large scale. . . . The variations of these two teeth always bear the same characters, in contradistinction to the second permanent molar, whose variability is of quite another nature" (2, pp. 99–101). Bolk failed to recognize the fact, however, that this similarity between the two teeth is not peculiar to man. In a recent paper (9), Gregory traced the similarity between the first permanent molar and the last deciduous molar back to the mammal-like reptiles at the base of the mammalian stem. For instance, the striking similarity between the third deciduous molar and the first permanent molar of the Middle Eocene Titanothere, *Palæosyops leidyi*, is illustrated in *fig. 2*. Another factor may be influential in effecting similarity between the last molar of the milk set and the first molar of the permanent set: there is a tendency for adjoining differentiated teeth to acquire the same anatomical characters. Gregory (10) refers to this phenomenon as *secondary polyisomerism*. I have discussed the widespread tendency to dental polyisomerism in man (13), and suggested that recent similarities in heretofore differentiated teeth may be due to some spreading genic or ontogenetic influences, or both. The likenesses would then be only evidence of the subjection of the tooth germ to forces that determine the number of primary parts or lobes of the tooth, and control rates of growth of these parts. Bolk's specimens also show that corresponding varia-

tions of milk and permanent molars consist of overdevelopment of one primary lobe.

Bolk's final argument on his first problem was based upon the occasional fourth molar in man. He claimed that it is an atavism. Many anatomists, as he pointed out, object to his theory on the ground that atavism would mean reversion to a hypothetical Mesozoic reptile having four molars. Bolk's theory obviates the necessity of such a daring application of the reversion hypothesis. He claimed that the human fourth molar is homologous with the third platyrrhine molar, which is already lost in the marmosets (*fig. 1*). "It is a well known fact," he wrote, "that a tooth which was lost in an earlier phase of phylogenetic evolution reappears sometimes as an individual variation of atavistic nature" (2, p. 101). Here, Bolk was inconsistent with himself. On pp. 105–106 he wrote: "It is clear that the main cause for the denial of the atavistic significance of the fourth molar is the fact that we must go back, until we reach the common ancestors of mammals, to encounter beings with an increased normal number of molars. And this objection is of great value, if a true one. . . . My conception of the manner in which man's denture originated from a platyrrhinically constructed ancestral form obviates all difficulties in a very simple and logical way. . . . The development of the fourth molar is also, in my opinion, an atavistic phenomenon. *But by my theory we are not obliged to descend to a problematical ancestor, who perhaps existed in the Mesozoic age and possessed a greater number of molars*" [italic not in original]. Yet, in discussing the paramolars he wrote (p. 122): "*And, considering that, save in some marsupials, the number of molars in all recent mammals does not surpass three, these paramolars evidently represent teeth which functioned in our ancestral forms of the mesozoic period*" [italic not in original]. These quotations require no comment.

Works in comparative anatomy and paleontology further weaken Bolk's contention. Selenka, Zuckerkandl, and Gregory, concur in the opinion that the fourth molar in man is a budding-off of the dental lamina—a progressive variation. "The fourth, and even the fifth molar when present, are in all probability neomorphs both in man and in anthropoids, and the various phyla of the man-anthropoid group are independently acquiring a new growth-power at the posterior end

of the dental lamina, as has happened in other groups of mammals (e.g., *Myrmecobius* among marsupials, sirenians, toothed whales, armadillos, *Otocyon*, etc., among placental mammals)" [Gregory (8, p. 467)]. Furthermore, Gregory (8, p. 233) has demonstrated the presence of the fourth molar is the platyrrhine genus *Ateles* (*fig. 3*). Bolk is also at variance with genetics. "The past is indestructible," said Dollo (5). What is lost is lost forever. Characters may persist as vestiges despite loss of function, such as tonsils, vermiform appendix, auricular muscles, etc. However, when they are lost for hundreds of thousands of generations the chances for reappearance are very slim.

Bolk's *second problem* is entited: "To which dentition do our molars belong?" (2, p. 107). In discussing this question, he maintained that the first permanent molar was originally a deciduous tooth. Second and third permanent molars, however, are elements of the permanent dentition. His treatment of the problem may be summarized as follows: (*a*) There are two types of supernumerary teeth in the molar region of man: "the additional element situated immediately behind the third molar" (2, p. 109) to which he referred as the *distomolar*; and "the supernumerary tooth—situated at the buccal side of the dental arch, either in the corner between the second and third molar, or in between the first and second" (2, p. 109). Bolk called these *paramolars*—the supernumerary element between first and second molars, *paramolar I*; that between second and third molars, *paramolar II*. (*b*) Distomolars and paramolars are distinct types. This statement is based on one of his specimens in which both are present in the same jaw. (*c*) Tubercles on the buccal side of molars are nothing more than paramolars coalesced with the normal tooth. He designated them as *paramolar cusps*. Sometimes the paramolar is connected, only at its neck, to the normal tooth. There are many intermediate forms between the free paramolar and the paramolar tubercle. (*d*) The paramolar cusp is never found on the first molar. It is always united with the anterior buccal cusp of second and third molars.

One cannot criticize these observations, but Bolk's deductions therefrom are questionable. As mentioned above, he regarded paramolars as homologues of some mesozoic ancestral form, after he had

previously objected to the idea of reversions to so distant a past. He said (2, p. 124): "I think the paramolars are the continuation of the elements of the milk dentition which, however, during the process of the evolution of the mammalian dentition, grew rudimentary and finally were eliminated from the row of functional teeth. Their reappearance in man must be ascribed to retrogression" (*fig. 1*). Here he opened his reversion theory to further criticism, for he cited the consistency in position of the additional elements as a proof of their atavistic nature. The paramolar cusp and root, he maintained, always appear on the buccal side of second and third molars; the distomolar cusp, always on the distal surface of the third molar. All of the specimens illustrated in Bolk's paper exhibit this uniformity of position. The teeth shown in *figs. 4, 5* and *6* are second and third molars from the collection at the Columbia University College of Dental and Oral Surgery. This collection, although smaller than the one described by Bolk, contains molars with extra cusps and roots on the mesial, lingual and distal surfaces, besides the buccal. The tubercle of Carabelli is on the side opposite to the paramolars. It frequently gives forth its own root. According to Bolk's scheme it has no ancient homologue. Paramolars I and II are supposed to be atavisms recalling primitive conditions, where M_2 and M_3 were preceded by deciduous teeth as shown in formula 1, *fig. 1*. Bolk said that the paramolars are homologues of these deciduous teeth. There is, however, no fossil evidence that any such animal ever existed. Such a dentition is purely hypothetical.

The *third problem*: "progressive variations in human dentition" (2, p. 131). Bolk divided anomalies into three categories: (*a*) variations of an atavistic nature; (*b*) variations of a progressive character; (*c*) ontogenetic deviations. The first group includes evolutionary regressions; the second group includes evolutionary progressions; the third group is for "variations in the structure of our denture which have nothing to do with its developmental history, either in the past or in the future" (2, p. 132).

Of the various types of *supernumerary incisors* he claimed (2, p. 133) that only that "arising close to the median line of the palate is an atavistic one" (*fig. 1*). The others are instances of "schizogenic variations. . . . because they originate by a division of one of the two

normal incisors. They are instances of reduplication without any historical significance" (2, p. 133). Since Bolk recognized the existence of a schizogenic supernumerary tooth, why cannot the fourth molar have such an origin? Here, again he was in serious contradiction with himself. On p. 104 he said: "The appearance of a fourth molar (additional molar) in man and apes is a wholly incomprehensible fact." He maintained that, if this variation occurred only in the orang, the "additional-molar" hypothesis might be correct, "for in this Anthropoid the third molar is always a strongly developed element" (p. 105). Bolk rejected the hypothesis because the fourth molar also appears in man where the posterior end of the jaw is "in a state of retrogression." In other words an additional element can only appear in a section of the jaw where there is a sturdy development of the teeth. However, he said (p. 134): "One of the most generally known indications of reduction in our denture is that of the second incisor in the upper jaw." On p. 133 he refuted his own arguments at the beginning of his paper: "The other supernumerary incisors, nearly always standing regularly in the arch, and in most instances possessing a normal incisiform crown, are not at all of an atavistic nature. They are instances of schizogenic variations, as I call them, because they originate by a division of one of the two normal incisors." Both molars and incisors show degeneration, yet for the first he rejected the "schizogenic" theory; for the latter, he advocated it. Bolk regarded the supernumerary incisor at the median line as an atavism because it is the most common. For the other supernumerary elements he had a different theory—a very unconvincing procedure.

The tendency to *loss of the lateral incisor* was regarded by Bolk as one of the most common of the "progressive variations." Early in the primate stem there were three incisors. Bolk noted that "the genus *Adapis* forms a link between the primitive and the recent condition, still possessing three incisors in the first, but only two in the second dentition" (2, p. 135). This would be interesting if true. But *Adapis* has only two incisors in the deciduous dentition. Bolk probably misinterpreted the illustration of the lower jaw of *Adapis parisiensis* in Stehlin's (11) monograph on the Adapidæ. From this diagram (*fig. 7*) it can readily be seen how the socket of the deciduous

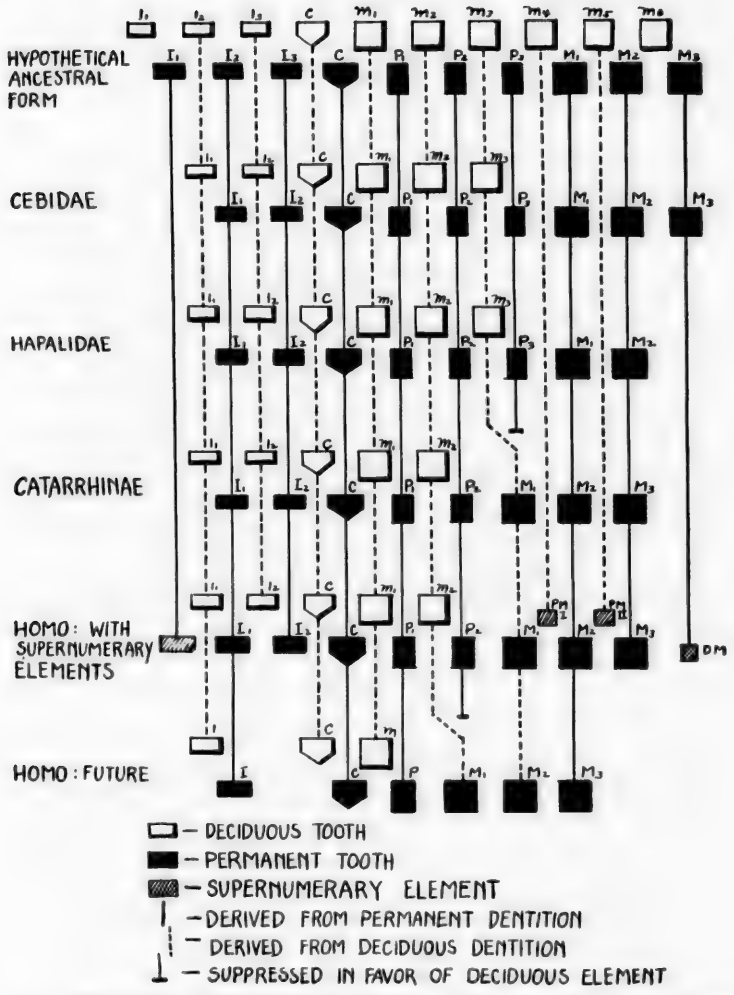


FIG. 1. Diagramatic representation of Bolk's theory of origin, evolution, and future of human dentition.

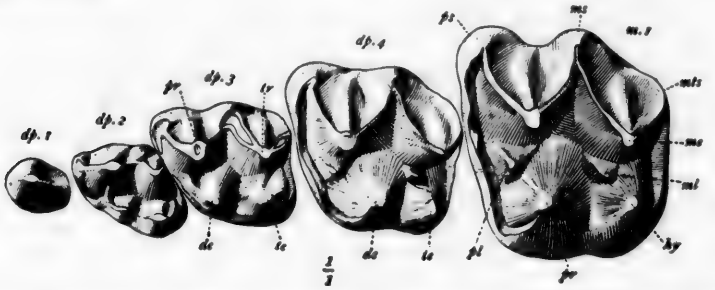


FIG. 2. Deciduous molars and first molar of *Palaeosyops leidyi*: Middle Eocene titanothere. (After Osborn)

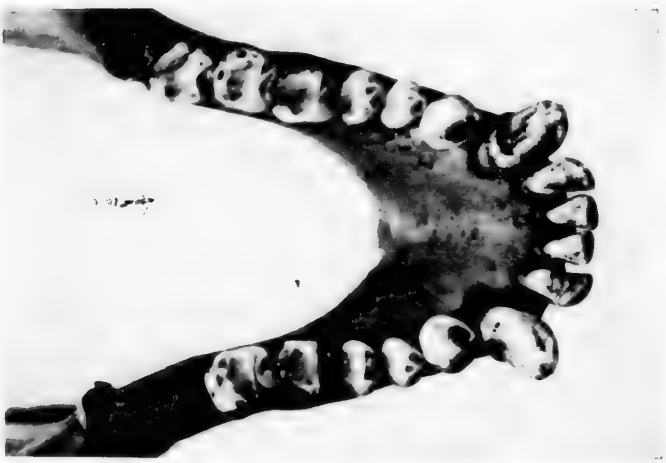
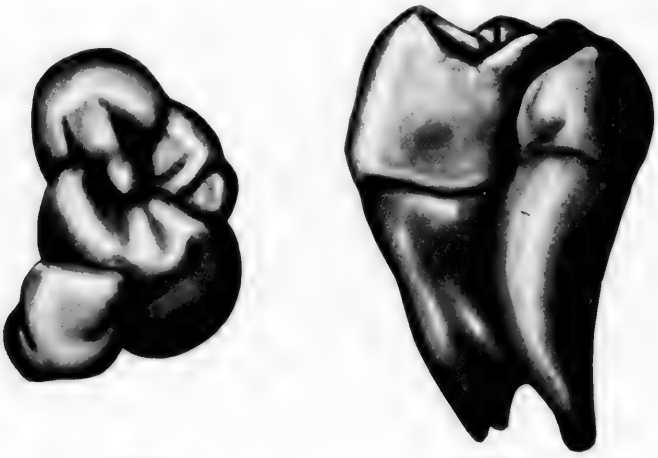
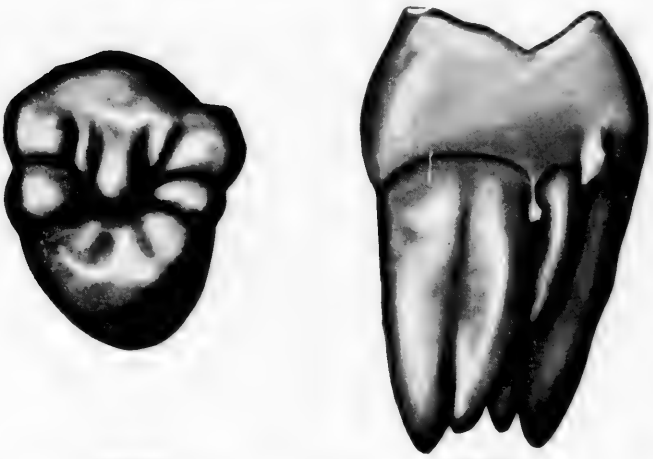


FIG. 3. Specimen of platyrrhine genus *Ateles*, with fourth molars in lower jaw. (After Gregory)



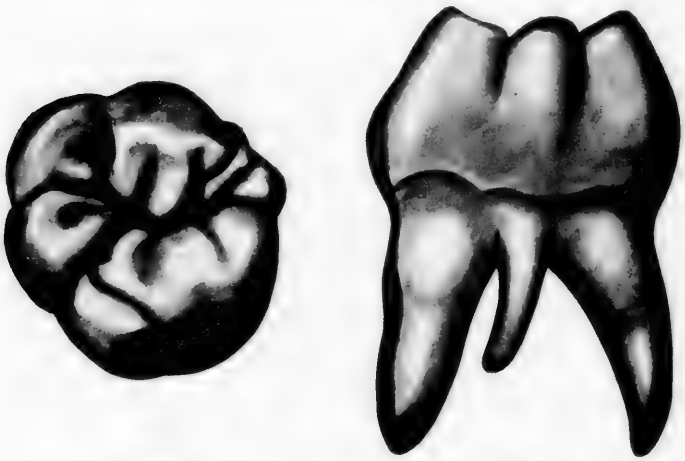
Occlusal Mesial

FIG. 4. Upper left third molar, with over-developed tubercle of Carabelli and extra root, showing occlusal (left) and mesial (right) aspects.



Occlusal Mesial

FIG. 5. Upper left third molar, with extra root and enamel pearls on mesial surface, showing occlusal (left) and mesial (right) aspects.



Occlusal Distal

FIG. 6. Upper right third molar, with extra root on distal surface, showing occlusal (left) and distal (right) aspects.

cuspid could be mistaken for that of a third incisor. This error has been made before. In 1927, deChardin (3) reported a species of *Omomys* with the alveoli of three incisors. The fact, however, that an early primate had three incisors does not prove that it was the incisor nearest the median line that was lost, nor that the recent supernumerary incisor is a reversion to the primitive condition of three incisors.

Another common progressive variation is *diminution and loss of the third molar*. Bolk felt that here he was presenting the cardinal evi-

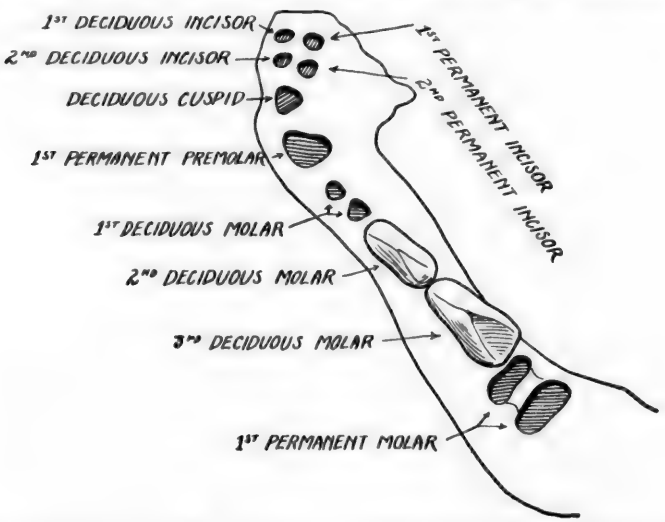


FIG. 7. Lower jaw of *Adapis parisiensis*. Socket of deciduous cuspid mistaken for that of third deciduous incisor. (After Stehlin)

dence in support of his theory. He arbitrarily linked loss of the third molar with occasional loss of the second premolar and consequent retention of the second deciduous molar. This, he assumed, was a repetition of earlier evolutionary history when the marmosets lost their third molars and the third premolar of the Catarrhinæ was suppressed. He offered an explanation for the relationship between disappearance of the third molar and retention of the second deciduous molar. "As the third molar diminishes and the grinding surface is shortened . . . . at the anterior end of the molar region the dentition

strives to compensate for the loss at the posterior end, for the grinding surface of the second milk molar is a larger one than that of the third premolar" (2, p. 139). "The recent phenomenon is a . . . . remarkable continuity in the developmental history." Although this last discussion was regarded by Bolk as conclusive proof, it actually discloses the fundamental fallacy in his theory. Reduction of the third molar, and loss of the second premolar with consequent retention of the second temporary molar, are not uncommon in man. Bolk undoubtedly knew of these two human dental variations before he devised his theory. He worked on the hypothesis that loss of the third molar and retention of m_2 were "methods" of evolution, and sought to find their prototypes in early primate history. But man is the only primate that shows both of them at the same time. He therefore pieced together different variations from widely separated species to create his transition theory. The taxonomic difficulties were solved by assigning new positions to the genera that were involved in his new scheme. The theory was used in turn to prove the new taxonomic relations. Finally, the human variations were used to prove the theory.

Bolk made no attempt to show why a compensatory increase in arch length is actually desirable from a mechanical standpoint, if the grinding area of the recent human dentition is reduced by disappearance of the third molar. Perhaps man no longer needs a large grinding area. Even if he did, by what genetic or evolutionary process does the change take place when the teeth no longer play a decisive part in the selection and adaptation of the human species? Bolk said that "the *dentition* strives to compensate for the loss." Like several other dental anatomists, he invented a creative force to perform at his will.

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DENS IN DENTE

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As indicated by Kronfeld (1), the first reference to *dens in dente* was made in 1859, by Sir John Tomes in his *System of Dental Surgery*. In the 1906-edition of this book (2), under the classification, "*odontomes coronaires* of Broca," the specimens described by Tomes and Salter are mentioned, but the general description is essentially as in the original edition. Since 1859, including Kronfeld's specimen, about fourteen *dens-in-dente* phenomena have been described. That this malformation is a rarity is evidenced by the fact that, despite almost universal use of the x-ray, few have been seen. The name *dens in dente*, while convenient, is not accurately descriptive. All of several types that have been adequately described and illustrated have proved, by the sequence of tissues, to be invaginations of various areas of the tooth. If Kirk's (3) specimen was actually an "inner tooth" with enamel covering, rather than lining, it is the only exception. Of the cases reviewed by Kronfeld, including his own, only two—those of Salter and Miller—were multiple-crown invaginations. The remainder, excepting those of De Jonge Cohen (4), were single invaginations of the coronal tissues. The cases of De Jonge Cohen were linear radicular invaginations in the mesio-lingual sulcus of lower bicuspids.

Since the growth of the tooth germ is regarded as essentially centrifugal in nature, it is plausible to conclude, with Kronfeld, that one or several points of retardation in growth of the coronal tissues and their subsequent involvement account for the coronal cases. On the basis of the influence of Hertwig's epithelial sheath in root-bifurcation processes, as indicated by Orban (5), we might account for the radicular invaginations as due to abnormal retardation, or to ingrowths of parts of the sheath, and the subsequent enclosure of these areas by unions of the projecting parts; in short, as aberrations of the root-bifurcation process.

Because of the rarity of *dens in dente*, an excellent specimen of the single coronal-invagination type recently seen in this laboratory may be worthy of mention. The tooth is an upper right lateral incisor from an Italian boy of 16. There was a history of labial sinus-infection and typical abscess of about six-months duration. The extraction

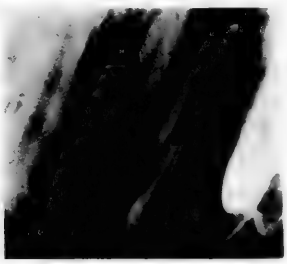

FIG. 1 FIG. 2 FIG. 3

FIG. 1. ORIGINAL RADIOGRAM OF CASE

FIG. 2. RADIOGRAM OF TOOTH AFTER EXTRACTION, IN APPROXIMATELY SAME POSITION OCCUPIED IN THE ARCH. (SLIGHTLY ENLARGED)

FIG. 3. RADIOGRAM OF TOOTH AFTER EXTRACTION. TAKEN FROM POSITION AT RIGHT ANGLES TO THAT USED IN *fig. 2*. (SLIGHTLY ENLARGED)

A

B

FIG. 4. LABIO-LINGUAL GROUND SECTION THROUGH LONG AXIS OF AUTHOR'S SPECIMEN
Spaces marked *A* and *B* are further magnified in *figs. 6 and 5*, respectively.

and original radiogram (*fig. 1*) were made by Dr. J. W. Tall, of Columbus, and given to Dr. I. A. Bottenhorn of the Dental College Faculty, who presented them to the Research Laboratory for examination. Grateful acknowledgment is made to both for this specimen. Inquiries made of Dr. Tall revealed the fact that the upper left

FIG. 5. DISTORTED CONDITION OF ENAMEL RODS AND PRESENCE OF TUFTS AND LAMELLAE IN INVAGINATED ENAMEL AREA (*B, fig. 4*)

C. O.—part of central opening through enamel communicating with pulp apically.

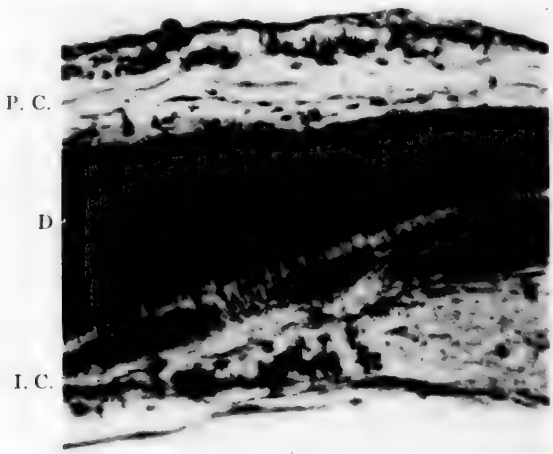

FIG. 6. ENLARGEMENT OF AREA INDICATED BY A IN *fig. 4*, SHOWING GROWTH OF CEMENTUM ON PULPAL SIDE OF DENTIN IN EFFORT TO STABILIZE PARTLY DETACHED ROOT-APEX

P. C.—peripheral cementum. *D*—dentin. *I. C.*—internal cementum.

lateral of this patient resembled externally the extracted tooth, but had no opening in the crown; and a radiogram showed no evidence of *dens in dente*.

The crown of the tooth resembled a cuspid rather than a lateral incisor. The radiogram of the tooth in place (*fig. 1*) indicates that it was in a position of clockwise torsiversion. A large area of rarefaction surrounded the root apex, and the tip of the root was raised mesially, leaving an opening on the distal side. Whether this opening was a large lateral foramen or due to resorption is uncertain; but, because of lack of cementum extending into it from the crown side, we are inclined to consider it a result of resorption. The *dens-in-dente* phenomenon in the tooth is clearly shown in the original radiogram The mesial and distal aspects of the tooth in clockwise torsiversion showed bilateral symmetry. On both lingual and labial sides of the crown were slight depressions, 2 to 3 mm. in width, and parallel to the long axis of the crown. One of these extended across the cemento-enamel junction onto the root. Both showed some blanching of enamel. At the extreme tip of the crown, situated about equidistant from the two depressions, was a small dark pit, the only sign of defect in the crown. The apical end of the root was tipped back like a lid with the opening toward the distal. The apical two-thirds of the root was enlarged and almost circular in cross section. The extracted tooth was radiographed directly on an x-ray film in approximately the position it had occupied in the patient's mouth (*fig. 2*). The reproductions of all three radiograms (*figs. 1, 2* and *3*) are somewhat enlarged. In *figs. 1* and *2* a small, intensely radiopaque, linear area in the tip of the crown is an explorer-point broken off in the small pit. This was the only external evidence of a break in the enamel. *Figs. 2* and *3* also show that narrow extensions of the overlarge pulpal space pass coronally around the root-half of the "inner tooth," while its coronal half is fused to the "outer tooth." The enamel of the "outer tooth" is continuous with that lining the "inner tooth." Both views also show a seeming failure of enamel continuity at the extreme apical end of the invagination. Unfortunately it was necessary to choose in the ground section between the pit opening in the crown and the opening from the invaginated end to the pulp. As the grinding was done the latter could be seen as a dark line, part of which

appears in *fig. 5* marked C.O.; it connected the pulp and the central-invagination area.

Since Kronfeld decalcified his specimen, with consequent loss of the enamel, we decided to grind ours. *Fig. 4* is a photograph of the ground section (×5). The enamel on the exterior of the crown is nearly normal in appearance, with fairly straight rods and few lamellae or tufts. There are frequent and well developed spindles in the two crown-tips. The involuted enamel has many lamellae and tufts, especially in the deeper part, as shown in *fig. 5*. The invaginated space contains no fibrous or osseous tissue, as did Kronfeld's specimen; neither does it show any cementum, as did the cases of Tomes and Miller (6). There is considerable evidence of enamel disintegration; in several places caries penetrated to the dentino-enamel junction and somewhat affected the dentin. The raised apical part of the root is covered by cementum, except on the superior surface, where active resorption of dentin was in progress. This cementum layer extends coronally along the pulpal wall of the dentin on the attached side, as shown in *fig. 6*, which is an enlarged view of the area marked *A* in the photograph of the ground section.

Summary. Another specimen of the rare tooth malformation, *dens in dente*, is presented. The case has been studied by means of radiograms; and, in order to preserve the enamel, by ground sections. The involuted enamel, by the irregularities shown, gives evidence of crowding and consequent pressure during development. The specimen supports the theory that such malformations represent retardations at one or more points of growth, resulting in invaginations of the tooth tissues.

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CATAPHORETIC VELOCITY AND LOCALIZATION OF STREPTOCOCCI ISOLATED FROM INFECTED TEETH OF PERSONS HAVING SYSTEMIC DISEASE[1]

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Despite voluminous literature indicating the importance, as sources of disease in remote structures, of mild, localized, and often symptomless infections—such as those commonly present in tonsils, teeth, prostate gland, and uterine cervix—there is need for further study of the problem, particularly from the dental standpoint. Proper clinical application of established truths, while seemingly simple, is often most difficult and calls for the combined judgment of physician, dentist, and specialist. In the matter of operative procedure in dealing with foci, especially removal of infected teeth, much needs to be learned.

The importance of pulpless, roentgenographically-positive teeth is now generally recognized, but that of roentgenographically-negative teeth as sources of infection in remote tissues is still unappreciated by members of the medical and dental professions. Intensive application of the principles involved in focal infection and elective localization have unfortunately sometimes led to insufficient consideration of other important factors in the pathogenesis, diagnosis, and treatment of disease. Infected tonsils and teeth are being removed for the cure of conditions in which there is no possibility of causal relationship; and, inadvertently or otherwise, they are allowed to remain indefinitely in cases in which disease unquestionably, or with a high degree of probability, is attributable to or is made worse by localized infection in these or similar structures. Many clinicians still regard recurring symptoms of acute infection in these structures as prerequisites of a possible source of systemic disease. They seem not sufficiently aware of the

[1] Read at the meeting of the First and Second District Dental Societies of the State of New York (Centennial of the Organization of the First Dental Society), New York City, December 3–7, 1934.

fact that the streptococcus isolated from symptomless foci, and with which respective diseases have been reproduced, usually have low general virulence. Exacerbations following attempts at removal of certain foci of infection (especially infected teeth) for the relief of systemic conditions are still too often considered as indicating that the idea is wrong instead of as proof that the responsible focus has been found, and that the methods employed were inadequate. Too much is often expected from removal of foci, especially in far advanced, chronic conditions. Although a focus of infection which for mechanical reasons cannot heal, or drain to the surface, makes for a prolonged conflict between host and parasite—resulting often in disease in remote structures, as indicated clinically (2, 7, 11, 13, 14, 29, 31) and as proved experimentally (2, 5, 10, 12, 17, 27, 28)—there is general lack of appreciation of the importance of this basic fact. Moreover, this forced relationship is considered generally in a too mechanical sense. Bacteria and their toxic products absorbed from a focus of infection must overcome the same resistance of the host as those absorbed from regions other than foci in a grossly mechanical sense and which, although less likely, may also result in systemic disease. It has been shown conclusively that pulpless teeth (1, 3, 4, 5, 6, 9, 16), whether roentgenographically positive or negative, of persons who are ill with systemic disease, are nearly always infected, whereas normal vital teeth are nearly always sterile.

Experiments on dogs—in which elective localization and specific disease, and concomitantly increased susceptibility to intercurrent infections, followed devitalization and infection of teeth—leave no doubt of the basic fact that infected teeth may be a source of systemic disease and of general lowered resistance (18, 23). Nephrolithiasis, alkaline phosphatic cystitis, ulcer of the stomach, ulcerative colitis, heart disease, and chorea-like movements have been reproduced consistently by removing the pulps of teeth of dogs and infecting the pulp chambers with streptococci isolated from teeth or other organs of patients suffering from these respective diseases. Housing conditions and a diet adequate to keep the control dogs well, proved inadequate for dogs that were made to harbor the type of dental infection so commonly found among patients suffering from the diseases named, or other diseases. The dog teeth from which the pulps had been re-

moved, and the pulp chambers infected, became discolored, but remained firmly and painlessly in place. In most instances variable degrees of periapical rarefaction and deposit of granulation tissue developed, from which the streptococcus that had retained elective localizing power was consistently isolated and demonstrated microscopically. While localization and production of lesions in remote structures, aside from predisposing factors such as trauma, are attributable largely to inherent or acquired, highly labile, specific properties of bacteria and their toxins found in foci, there is lack of knowledge as to how bacteria, usually streptococci, maintain or acquire their respective specificities, and to what properties peculiar localizing power is attributable. The fact that bacteria and their toxic products do localize and produce lesions specifically in certain structures is no more remarkable than specific pharmacologic action of certain drugs, and the basic reasons are no doubt similar. In a recent paper (23) I reported a general summary of experimental studies on focal infection and elective localization, and that of recent corroborative work by others (8), in which references to the more important publications are given. In the present paper I report results of studies, by new methods, on the specificity of streptococci from infected teeth of patients suffering from systemic disease and, so far as possible, indicate the epidemiologic and etiologic importance of the newly ascertained facts.

Methods. The material for study was obtained chiefly from patients in the Mayo Clinic, and Rochester State Hospital. The diagnoses were made by attending physicians. Teeth were extracted, with aseptic precautions, by members of the Section on Dental Surgery. Nasopharyngeal swabbings were made by me. The field of operation, after block anesthesia with procain and epinephrine, was isolated with sterile cotton and gauze. The mucous membrane was thoroughly disinfected with tincture of iodine and alcohol. A control culture, in dextrose-brain broth, of the swabbing of the disinfected area was made just before extraction forceps were applied. The tooth was then extracted and the apical third, usually of a tooth with a single root with or without adherent granuloma, was immediately severed with bone-cutting forceps and placed in 2 cc. of sterile gelatin (0.2 percent)-Locke solution containing a small amount of quartz sand. This was taken to the laboratory where, after thorough shaking, cultures were made as

follows: Three drops of the washings were spread over the surface of a blood-agar plate. About 0.5 cc. was inoculated into melted and cooled (40°C.) soft dextrose (0.2 percent)-brain agar (0.5 percent) in tall tubes, and 1 cc. inoculated chiefly around the pieces of brain at the bottom of a tall warmed (35°C.) tube of dextrose (0.2 percent)-brain broth. The remaining gelatin-Locke solution containing the root tip was then transferred to an additional tube of warmed dextrose-brain broth. In special instances the root tip was wrapped in sterile gauze, crushed in the jaws of a vise, and the pieces placed in dextrose-brain broth, or dextrose-brain broth to which 10 percent sterile ascitic fluid had been added. One of the tubes of dextrose-brain broth was routinely heated to the boiling point to reduce the free oxygen, and was then cooled just before adding the inoculum.

The nasopharynx was swabbed, without touching the tongue, with cotton-wrapped aluminum wires bent to a suitable angle. The swab was immediately placed in 2 cc. of Locke solution containing gelatin (0.2 percent), and the material contained on the cotton washed off by vigorous agitation. Cultures of the washings were made on blood-agar and, routinely, into previously warmed dextrose-brain broth. The dextrose-brain broth used throughout the study was either freshly prepared or not older than a week or ten days. In general, the fresher the broth the better the results. Cultures were usually made in the afternoon and the tubes incubated at 35°C. until the following morning, or longer if abundant growth had not occurred. The following morning plate cultures and smears were made of all positive dextrose-brain broth cultures. Only those showing pure or nearly pure cultures of streptococci in smears, the control cultures for which were negative, were used for cataphoretic studies.

Dextrose-brain broth was prepared by dissolving 8 gm. of dehydrated "Bacto" broth in 1,000 cc. of distilled water, and adding 8 gm. of NaCl, 2 gm. of chemically pure dextrose, 1.5 cc. of *N* NaOH, and, after cooling, 1.0 cc. of Andrade's indicator. It was also prepared from beef infusion, adding 0.2 percent dextrose in the usual way and titrating to pH 7.4. Several bacteriologic peptones on the market have been used with about equally good results. The dextrose-brain agar was prepared from the broth by adding 4 or 5 gm. of powdered agar to each liter—just enough to cause it to set. The media were placed in tubes measuring 20 by 1.5 cm. To each tube were added three pieces of calf or beef brain, each about 1 cc., and two or three pieces of crushed marble ($CaCO_3$) to insure correct reaction. The column in each tube, after adding brain and marble, was about 12 cm. high. The media were sterilized for twenty minutes in an autoclave at a pressure of 20 pounds. Growth in these media, which were

kept at 35° to 37°C., usually occurred within twenty-four hours; in most instances, especially when the cultures were from pulpless teeth, it began at the bottom of the tube and forced its way to the top as oxygen was consumed. This usually occurred within twenty-four hours in the dextrose-brain broth; in the dextrose-brain agar growth to the top, or more often to within 1 cm. of it, frequently took from two to three days. Negative cultures were observed daily for a week or ten days before being discarded. Colonies in the agar may be exceedingly small; or may develop only around pieces of brain at the bottom and easily be overlooked; or may be so numerous as to give the appearance of a negative culture.

As a routine for cataphoretic studies, the top 2 cc. of the primary 16 to 24-hr. culture in dextrose-brain broth—and, in some instances, of rapidly repeated subcultures or of single-colony cultures from dextrose-brain agar, in this medium as isolated from the tooth washing—was poured into scrupulously cleaned test tubes of the usual size. This was then centrifuged until the broth had just cleared, and the supernatant fluid poured off and thoroughly drained by leaving the tubes inverted on sterile filter paper or on a towel for at least five minutes. About 2 cc. of distilled water was added and the sedimented bacteria thoroughly mixed with the water; then, about 10 cc. of distilled water was added from a siphon and the content poured into a Northrop-Kunitz-Mudd cataphoresis apparatus. Age of cultures, duration of centrifugation, amount of water added to the suspension, time after making the suspension in distilled water, and readings, were made as nearly comparable as possible. All readings were made in the same apparatus, which was properly cleaned and checked by known suspensions, usually by the same person without knowledge of the source of the cultures. In the lower neutral layer of the cell, the velocity of twenty suspended streptococci was determined, using a stop-watch to measure the time required for the organisms to traverse the unit distance of 48.1 microns, or two squares in the field. The direct current of the zinc sulphate, non-polarizable electrodes (122 volts), and the temperature (26°C.), varied little. The effective voltage between the platinum electrodes (which were 3.79 cm. apart) by actual measurement in a large number of control preparations of suspensions of streptococci from different diseases, determined immediately before and after taking the readings, was about 26.5 volts; hence the velocity in microns per second, per volt per cm. equals:

$$\frac{48.1}{\text{time} \times \dfrac{\text{voltage}}{\text{distance between electrodes}}}$$

For example, neurotropic streptococci traversed the unit distance of 48.1 microns in four seconds when an effective potential difference of 26.5 volts is measured between the electrodes separated by a distance of 3.79 cm. Hence the mobility of these organisms in microns per second, volts per cm., equals $\frac{48.1}{4 \times \frac{26.5}{3.79}} = 1.72$ microns per second, volt per cm.[2]

RESULTS. Detailed analysis of the clinical results following removal of foci in these cases, together with a larger series, is contemplated. Only a general statement will be given here. Improvement following removal of foci, in this series of cases having infected teeth—from which the apparently causative streptococci were isolated as in previous studies—occurred repeatedly (18, 23); so promptly in some cases, especially in those in which the disease was of short duration, as to leave little doubt that improvement was attributable to this cause. In this as in previous studies, it has happened repeatedly that removal of infected tonsils was not beneficial until one or more infected teeth also were removed. In some cases improvement occurred so gradually that it was impossible to decide whether it was the result of removal of infected teeth, of other therapeutic measures also employed, or of coincidental causes. In some cases the course of the disease was not perceptibly modified.

The results of studies of cultures from teeth have in part been reported elsewhere. In a great majority of instances, the control culture in dextrose-brain broth of the swabbing after sterilizing the field and just before applying the forceps remained sterile; and cultures in dextrose-brain broth and dextrose-brain agar, from washings of the root tip and from the root tip itself, nearly always yielded streptococci, chiefly in pure or nearly pure culture. Blood-agar platings of the cultures in dextrose-brain broth nearly always revealed pure or nearly pure cultures of green-producing, indifferent, or slightly hemolytic but never typically hemolytic, colonies of streptococci. Goodly numbers of staphylococcus-like colonies were only rarely encountered. The blood-agar plate inoculated with the washings remained sterile in about half of the cultures, and in the rest revealed relatively few

[2] I am indebted to Drs. Baldes and Pratt of the Section on Biophysics for checking the physical conditions of the cell.

colonies (always fewer than the number of colonies in shake cultures of dextrose-brain agar) of green-producing streptococci. The number of colonies that grew in shake cultures of the washings was large, a hundred or more in most instances. The blood-agar plate almost never showed growth when the number of colonies that grew in the shake culture in dextrose-brain agar was relatively small. Streptococci having characteristic cataphoretic velocities have also been isolated from the enamel organ of unerupted teeth, and even from trephined, apical ends of pulpless teeth which had previously shown rarefaction but in which there was filling-in of bone following antiseptic treatment (23).

The study of elective localization in animals, while justified by the great significance of the findings, is peculiarly exacting in its technical requirements, chiefly because of instability of the property on which elective localization depends; is time-consuming and expensive; and has other disadvantages. I have, therefore, sought out new methods that might yield similarly important information. After the suggestion of Dr. L. B. Jensen, he and I studied, cataphoretically, streptococci that had different elective-localizing power (24, 25, 26). We found (*a*) that a high percentage of streptococci from foci of infection, in cases in which patients suffered from encephalitis and allied diseases of the nervous system, had a "neurotropic" cataphoretic time and velocity of chiefly 3.45 and 1.72 microns per second, volt per cm., and that a high percentage of streptococci similarly isolated from cases of arthritis had an "arthrotropic" cataphoretic time and velocity of 2.30 microns per second, volt per cm.; (*b*) that there was definite correlation between elective-localizing power and the electro-cataphoretic velocities exhibited by the respective streptococci; (*c*) that dextrose-brain broth, which has been found so useful in a study of the bacteriology of foci and remote tissues, and in preserving the property on which elective localization depends, also maintained the characteristic cataphoretic velocity of streptococci; and (*d*), with Drs. Pratt and Sheard (15) of the Division of Biophysics, that if the charge or cataphoretic velocity of the streptococci was changed artificially by exposure to the high frequency field, short-wavelength electrical energy, there occurred concomitantly what I interpreted to be a change in localizing power. Thus, streptococci isolated from cases of chronic

infectious arthritis had marked affinity for the joints of animals which had received injections and had a markedly "arthrotropic" cataphoretic velocity (19); that is, a high percentage of them had a velocity of 2.3 microns per second, volt per cm. Streptococci isolated similarly from cases of chronic encephalitis had little or no affinity for joints, but had a marked affinity for the brains of animals which had received injections, and a markedly "neurotropic" cataphoretic velocity (26); that is, a high percentage of them had a velocity of 3.45 or 1.72 microns per second, volt per cm. When the cataphoretic velocity of "arthrotropic" streptococci isolated from cases of arthritis was changed to a "neurotropic" cataphoretic velocity by proper dosage of short-wavelength electrical energy, they lost what I interpret as affinity for joints, and concomitantly acquired affinity for brain; and when the "neurotropic" cataphoretic velocity of streptococci isolated from cases of encephalitis was changed to an "arthrotropic" cataphoretic velocity, they lost affinity for brain and acquired affinity for joints.

In *fig. 1* results of cataphoretic studies of streptococci from teeth of persons suffering from different diseases are given graphically. The distribution curves of cataphoretic time and velocity of streptococci are very similar and markedly "neurotropic" for the organisms from persons who had symptoms referable to the nervous system, and these curves are strikingly different from the distribution curves of streptococci from persons who were suffering from chronic infectious arthritis, which is markedly "arthrotropic." The distribution curves of cataphoretic velocity of streptococci isolated from teeth of persons who had miscellaneous diseases, which clearly were not attributable to streptococci, and of normal controls, is similar to that for streptococci isolated from patients who had arthritis, except that the percentage of organisms in the "arthrotropic" column (2.30 microns per second, volt per cm.) is not so high. The distribution curve of cataphoretic velocity of streptococci isolated from persons with neuromyositis is transitional, varying between that markedly "neurotropic," as for organisms from persons who had diseases of the nervous system, and that markedly "arthrotropic," as for organisms isolated from persons who had chronic infectious arthritis. The distribution curve of cataphoretic velocity of streptococci isolated from persons with

ulcer is moderately "neurotropic," which is in accord with the neurogenic theory of ulcer and with the fact that the site of disease, the mucous membrane of the stomach or duodenum (like the nervous system), is embryologically of ectodermal origin.

To check the reliability of the methods, the cataphoretic velocity of cultures of streptococci which grew from the tooth washing and from the root tip of the same tooth were determined simultaneously in 21 cases. Agreement in type of distribution of cataphoretic velocity was found in 20 and disagreement in only 1. In 47 cases, which are not included in *fig. 1*, the cataphoretic velocity of streptococci from extracted pulpless teeth and from the nasopharynx, was determined. Agreement in type of distribution of cataphoretic velocity occurred in 44 cases; disagreement, in 3. The average distribution curves for streptococci from teeth and nasopharynx, respectively, of the different groups of diseases, are given in *fig. 2*. The similarity between the cataphoretic velocities of streptococci isolated from teeth and nasopharynx in each group is striking; and each of these curves resembles closely that of the respective group in *fig. 1*.

Shifts in the distribution curves of cataphoretic velocity of streptococci isolated from nasopharynges of well persons and of persons ill with chronic systemic disease, according to season—and shifts toward that distribution curve characteristic of streptococci of epidemic respiratory infections, especially influenza—have been shown to occur with striking frequency (20, 21). In a few instances the slow toxigenic type of distribution curve characteristic of streptococci of influenza has been noted for streptococci isolated during epidemics of influenza from infected teeth of persons who did not have influenza, and also during convalescence from influenza (23). Cultures from teeth in the groups studied (*fig. 1*) were obtained over a period of four years. The cases in which cultures from teeth were made for each group of diseases were then divided according to whether the cultures were made during the six months from January to June, when respiratory infections were common—such as influenza, associated with streptococci having a markedly different distribution curve from that of streptococci isolated from normal persons—or during the six months from July to December, when respiratory infections, associated with streptococci having distribution curves

markedly different from the normal, occurred rarely. A decidedly slower, more "influenzal" type of cataphoretic velocity of streptococci was obtained for each of the groups of disease during the six months from January to June, when influenzal respiratory infections were most prevalent, than that of streptococci isolated in the period from July to December (*fig. 1*). Aside from this somewhat slower average

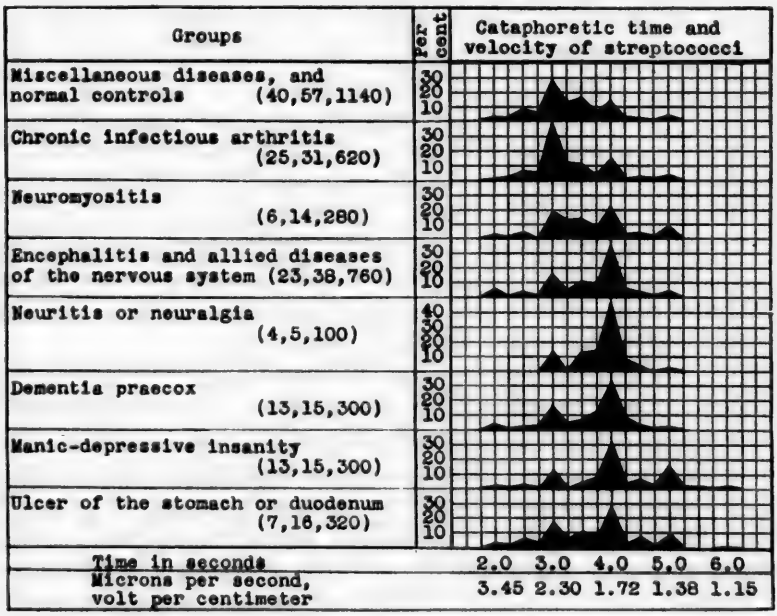

FIG. 1. Cataphoretic time and velocity of streptococci isolated from infected teeth of persons who have systemic disease. Numerals in parenthesis indicate, respectively, number of strains, cultures, and streptococci timed in each group.

velocity during the winter and spring months, and "arthrotropic" type of cataphoretic velocity of streptococci isolated during the period from July to December from persons who had neuromyositis, the distribution curves are fairly characteristic of the diseases in each group.

The data in *fig. 3*, for streptococci from nasopharynges of persons during acute attacks of nasopharyngitis (colds) or influenza, according

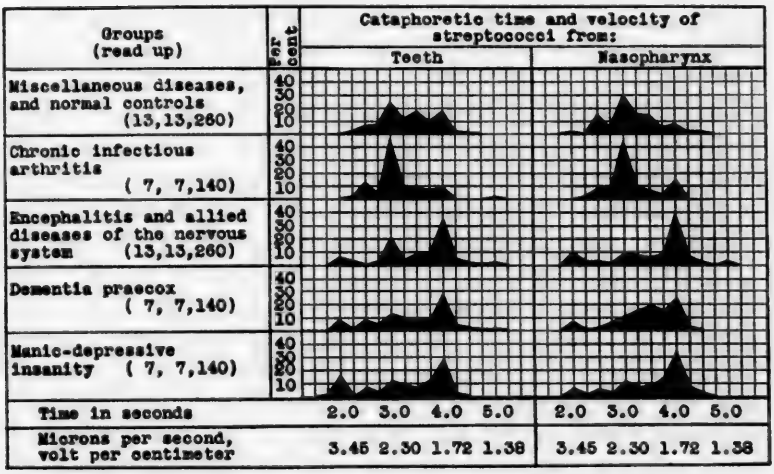

FIG. 2. Cataphoretic time and velocity of streptococci isolated both from infected teeth and nasopharynges of persons who have systemic disease. Numerals in parenthesis indicate, respectively, number of strains, cultures, and streptococci timed in each group.

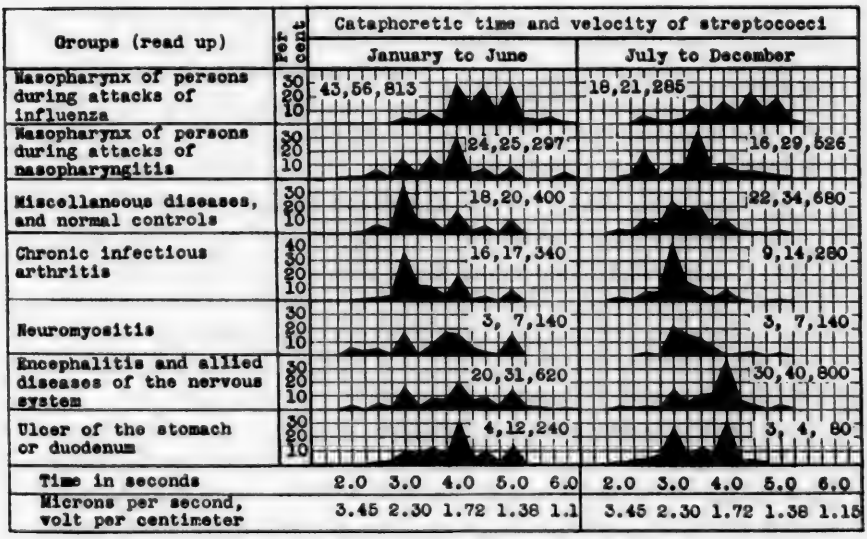

FIG. 3. Seasonal variation in cataphoretic time and velocity of streptococci isolated from infected teeth of persons who have systemic disease. Numerals in the graphs indicate, respectively, number of strains, cultures, and streptococci timed in each group.

to season, show that the distribution curves of cataphoretic velocity of streptococci isolated in cases of epidemic nasopharyngitis (common cold), and of streptococci isolated from patients with epidemic influenza, are strikingly different. No decided change in the average distribution curve of cataphoretic velocity in a study such as this should be expected during epidemics of colds, because the cataphoretic velocity (2.79 and 1.97 microns per second, volt per cm.) of streptococci from persons who have colds is not sufficiently different from the average cataphoretic velocity of streptococci from well persons and from persons who had the diseases studied. However, during epidemics of influenza there was a definite shift, in cataphoretic velocity of the streptococci from well persons and from persons ill with some chronic disease, toward that velocity characteristic of streptococci from persons with influenza (1.72, 1.54 and 1.38 microns per second, volt per cm.). The slower, more influenzal or "toxigenic" type of velocity, shown during winter and spring by streptococci from teeth in each group studied, was also noted of streptococci from the nasopharynges of persons who had epidemic nasopharyngitis (common cold) and influenza, respectively (*fig. 3*). These differences were not attributable to difference in the average temperature at which measurements were made in either period of six months. Of 191 cultures of streptococci that were timed, and which had been isolated from teeth, the organisms in 24 cultures had a "cold" or "nasopharyngotropic" type of velocity; in 22, an "influenzal" or "bronchotropic" type. These had a markedly seasonal distribution, corresponding roughly to that of the common cold and of influenza. Thus, of 24 cultures in which the organisms had a "cold" type of velocity, 18 were obtained during autumn, 1 during winter, 2 during spring, and 3 during summer. Of 22 cultures of organisms having an "influenzal" type of velocity, 2 were obtained during autumn, 10 during winter, and 10 during spring.

In previous studies of chronic diseases (19, 26), as in this new series of cases, specific slowing (charge-reducing) action of the serum of persons on streptococci from respective foci other than in teeth was noted. Similar specific action was found, in this study of serum of persons suffering from encephalitis, arthritis, hemiplegia, and ulcer, on the respective streptococci from teeth. Decided slowing action of

serum on streptococci was noted consistently for organisms from teeth of the respective patients, and on stock strains of streptococci from nasopharynges or teeth of patients who had the same disease, when the serum was in dilutions as high as 1 to 2,560. In contrast, slowing action of serum, in dilutions of 1 to 80 or 1 to 160, on streptococci did not occur when tested on organisms from persons who had diseases other than that from which the respective patient was suffering. Improvement following removal of teeth was especially noticeable in cases in which the specific-antibody content of the serum was high.

COMMENT AND CONCLUSIONS. The results of a study by cataphoresis of streptococci from infected teeth of persons suffering from widely different systemic diseases are reported. In this as in former studies, relief of symptoms occurred in some cases, including insanity (qualified in the next paragraph), following removal of teeth shown to be heavily infected, especially in cases in which the serum was rich in specific antibodies. Streptococci having peculiar cataphoretic velocities were isolated from pulpless teeth whether roentgenographically positive or negative, and also regardless of method of treatment to prevent or eradicate infection following the removal or spontaneous death of pulps. The methods leave no doubt that, in at least a great majority of cases, the isolated streptococci were from the apices of extracted pulpless teeth and were not contaminations. The high incidence of positive streptococcal cultures is attributable largely, it is believed, to the use of the dextrose-brain broth and dextrose-brain agar. These media afford a gradient of oxygen tension and other conditions favorable to growth of highly sensitive, partial tension or anaerobic organisms that for a time maintain characteristic cataphoretic velocity. In this study of cataphoretic velocity of streptococci from teeth it was found, as in the case of streptococci from other sources, that while characteristic cataphoretic velocity was retained for a longer time in dextrose-brain broth and dextrose-brain agar than in other media—especially if subcultures were made at frequent intervals—characteristic velocity, just as elective-localizing power, usually disappeared on prolonged cultivation on artificial media. "Specific" strains, irrespective of the disease in which isolated, frequently acquired distribution curves of cataphoretic velocity like that characteristic of streptococci from well persons and from persons who are not ill from streptococcal infections at times when there are no epidemics (22).

Streptococci from pulpless teeth and nasopharynges had similar distribution curves of cataphoretic velocity, characteristic of the disease from which the respective patient was suffering. It is not concluded from these results that manic-depressive insanity and dementia praecox were caused by the streptococci isolated. The fact that streptococci in such diseases are cataphoretically like those isolated in encephalitis and other diseases of the nervous system is suggestive. This question is now being studied.

The type of distribution curve of cataphoretic velocity of streptococci from well persons is similar to that of streptococci from persons who have chronic infectious arthritis—except that the number of streptococci having "arthrotropic" cataphoretic velocity is not so high. This fact is in accord (a) with the common occurrence of symptoms referable to joints and frank arthritis of human beings and (b) with the fact that the incidence of lesions in joints of animals following intravenous injection of streptococci from seemingly well persons has been found in repeated studies to be relatively high (30), although in our studies always less than the incidence of lesions in joints following injection of streptococci similarly isolated from persons having arthritis.

The distribution curves of cataphoretic velocity of streptococci from teeth of persons who have widely different diseases, and from well persons, vary perceptibly according to season and during epidemics of respiratory infection. The distribution curves of cataphoretic velocity, although remaining fairly characteristic of the respective diseases—and of the normal flora of well persons and of those not suffering from diseases attributable to streptococci during epidemics—become more or less like those characteristic of streptococci at hand in the prevailing epidemic disease, especially influenza. The results of a study of the cataphoresis of streptococci from human subjects, and the specific slowing (charge-reducing) action of respective sera, are corroborative of previous studies on elective localization, and emphasize anew the importance of variability in streptococci and of localized infection—especially in pulpless teeth—as sources of systemic disease. The focus affords conditions favorable for abundant growth and for forced entrance of bacteria and their products; and the peculiar localizing power and other properties of the bacteria, inherent or

acquired—of which cataphoretic velocity, or electrical charge, is expressive or a part—determine the site of systemic localization. The marked specificity of the causative streptococci—so clearly revealed by the new methods, and general clinical experience—indicates that while elimination of foci is of basic importance, other measures, such as active and passive immunization with specific vaccines and sera, are necessary to cope successfully with these and other diseases which are directly or indirectly attributable to streptococci.

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